

From Intelsat to Aerosat

REPRESENTATIVES of fifty-five countries turned up at the State Department in Washington last Friday to sign the inter-governmental agreement creating the new international communications satellite organization, Intelsat. The name of Intelsat, of course, has been wafting about for about seven years but it was referred to the temporary consortium, set up in 1964, to make a commercial global network out of communications satellites which at that time were merely in the experimental stages. The new Intelsat, unlike the old, will have juridical personality and an international executive. Yet the seven years of Intelsat's interim life were not happy ones. The practical success in putting geostationary satellites into service over the Atlantic, Pacific and Indian Oceans has been soured by the bitterness over the way that the organization representing the United States, Comsat, the communications satellite corporation, dominated the consortium. The European industrial nations who were among the participants of the original agreement never won anything like the share of the contracts that they had once hoped for. Of the dozens of costly Earth stations built around the world to work with the system, for which non-American suppliers were more readily available than for the satellites themselves, the great majority were built by American companies. So great was the resentment over this and over the American antagonism toward regional communications satellites outside the Intelsat system, that France, one of the original signers of the 1964 agreements, did not sign last week. Neither did Belgium, Mexico and West Germany, although all may at a later time.

In the new Intelsat the American influence is considerably reduced. Under the old arrangements, the United States held a majority of the voting shares and could not slip below 50.4 per cent. Britain was a poor second with less than 10 per cent (the percentages varied as new members—eventually 80—joined Intelsat). But the American dominance went beyond that. Comsat was the autonomous manager of the system and as such was not very forthcoming about the reasons and financial details about the contracts which it prepared on Intelsat's behalf. Both the American State Department and Comsat were dismayed to find, as the negotiations for a permanent agreement began, that Britain joined with the Europeans in fighting for a more internationally balanced organization.

That has been achieved but it is pyrrhic victory. In the first place, three expensive, tedious and acrimonious international conferences were needed to reach the two agreements (one for governments, one for telecommunications entities like Comsat and the British Post Office) which were opened for signature last week. In the second place, the organization that has resulted from all the wrangles and compromises is over-structured—over-defined to the point where it will be weak and inflexible in considering the new services which communications satellites may offer in the late 1970s and early 1980s.

The new Intelsat is to have four parts. The most powerful will be the Board of Governors on which the biggest investors will be represented, with their votes weighted according to the size of their investment. One

restriction on Comsat has been built in here, for no country may have more than 40 per cent of the vote. The other significant thing about the board is that its representatives will be telecommunications men, which will severely limit Intelsat's capacity to develop satellites for air navigation, weather mapping or any kind of communications except public telecommunications services. Another part will be the Assembly, where each member country will have one vote. The Assembly will have no power to make decisions or to dictate to the Board of Governors. All it can do is to make recommendations about general policy—a victory for the United States and a defeat for the developing countries, which wanted a strong Assembly as they will have little or no voice in the Board of Governors. There will also be an international directorate to handle the day-to-day affairs of the organization. Nothing like this existed under the temporary arrangements. The directorate will be headed for the six years after the agreement comes into force by a secretary general, whose function will be to monitor Comsat in its performance as manager. Then after six years, he will be superseded by a director general.

The fourth part of Intelsat is the management, which handles the practical operations of the system. The contentious question—will Comsat continue or not?—has effectively been shelved for six years. Comsat will continue for that period but under a contract limiting its tenure and then by 1978 must apply for the job together with any other organization which may present itself. Comsat is by no means certain of holding on. A European aerospace consortium might by then be qualified to manage a satellite system. The Hughes Aircraft Company, the world's major satellite builder, would qualify now; so would the National Aeronautics and Space Administration. Thus the fights over management seem doomed to be prolonged and renewed during the very years when Intelsat, as it tries to pull itself together as a fully-fledged autonomous organization, should be coming to grips with more important questions, such as persuading the Russians to join, at least to agree to terms of practical cooperation between Intelsat and its own proposed Intersputnik international satellite system.

Just how limited Intelsat is in scope was shown by the agreement reached in Madrid earlier this month on an experimental aeronautical satellite system. The project was agreed on and will be carried out by the American Federal Aviation Administration and the European Space Research Organization and other participants. The plans are firm and clear: a pre-operational network of four geostationary satellites by 1974 with the aim of a fully operational network by 1980. It is hoped that a formal memorandum of understanding will be signed by November. Not only will the experiment have nothing to do with Intelsat, it will not even use Intelsat as a model. (Great hopes had been fixed upon Intelsat as a model for international commercial technological cooperation.) In fact, it will be at some pains to avoid Intelsat's mistakes.

A council called Aerosat is to be formed, composed of an equal number of representatives of the United States and Europe, and one representative from the government



of each of the other interested countries (Australia, Japan and the Philippines). At the same time, there will be an executive office with its staff equally balanced internationally. The council will attempt to prepare a final document proposing the specifications of the desired satellite. There is no question but that the satellites will use the UHF band, something that both the FAA and ESRO have agreed on, to the chagrin of commercial international airlines who would prefer a less costly VHF system. The money—about \$150 million—will be provided by the participating governments.

The advantages will be great. Various governments

will not have to bear the expense of proceeding with aeronautical satellite experiments separately and perhaps incompatibly. Eventually separation between aircraft flying routes such as the heavily congested North Atlantic will be substantially reduced. The present methods, in which aircraft cannot be pinpointed more accurately than to within ten miles, requires a horizontal separation of 120 nautical miles and a vertical separation of 2,000 feet. The specifications laid down for the experimental project would permit aircraft to be placed within one half mile of their actual position and to fly as close as 30 nautical miles apart, separated vertically by 1,000 feet.

What Market for Computers?

THESE have been bad times for computer manufacturers in Britain, those owned by British interests and those which operate in Britain as subsidiaries of parent companies in the United States. For several weeks now, there has been a steady stream of news of how computer manufacturers have been shedding their labour forces. A week ago, the Department of Trade and Industry provided statistics to show how sales of computers have declined both in Britain and in the British export market. The decline of business has been dramatic. The total deliveries of computers from British manufacturers declined between the last quarter of 1970 and the first quarter of 1971 from £79 million to £64 million, a decrease of 18 per cent. This is more than a seasonal effect. Deliveries of computers have risen steadily, quarter by quarter, since 1966 but appear to have reached a peak of £85 million in the third quarter of 1970. The decline in the export business has been particularly sharp. Home deliveries fell by ten per cent between the last quarter in 1970 and the first quarter in 1971, but export deliveries fell by no less than 33 per cent. In practice, roughly half the lost market consists of machinery which is assembled in Britain from components imported from elsewhere. There was actually a small increase of six per cent in the value of British-made computers delivered to the British market. And if the manufacturers are cast down by their statistics for equipment delivered, they must be even more depressed by the order books, which have been falling since the beginning of 1970 and which, by the beginning of this year, had become so meagre that there is bound to be a further decline in sales as this year wears on.

What has gone wrong? Why has the great fashion for computers been undermined so quickly? In the past few weeks, it has been frequently suggested that the potential purchasers of computing machinery have been discouraged by their own experience of the failure of computers to function properly, or at least as well as the prospectuses for them claim. Is it the case that as with other technical innovations of the past few years—nuclear power, for example—the usefulness of computing machinery has been diminished by optimistic and unattainable promises of what these innovations might accomplish? By all accounts, there is a good deal in this view. After all, the British experience in the past few months is only a more dramatic representation of what is happening elsewhere, particularly in the United States. Small and smallish companies of all kinds are everywhere to be heard complaining that the machinery which they have installed in

the past few years has failed to do the job required of it. Core storage turns out to be too small for what it has to remember. Processors turn out to be much slower in practice than they are in theory. The costs of producing software, in any case, substantially incalculable, turn out to be formidably high. And all too often the purchasers of computing machinery discover that their new computer does not help them to reduce the costs of operations or even to undertake fast clerking operations without anxiety.

Salesmen are not solely to blame, although many companies have done themselves a lot of harm by over-selling. There are, however, serious problems of a technical character. Computer people are the first to say how important is the systems approach to practical problems. For an innocent company about to buy a computer for itself, what this implies is that the potential customer has—or should have—a clear idea of what he would like the machine to do but no great concern about the precise manner in which it will function. To be sure, there is much in the view that one of the uncovenanted benefits of acquiring a computer is that it compels those who would use it to understand the business in which they are engaged. But there are limits to what the systems analysts are entitled to claim in this respect. After all, the installation of a new computer system is not an end in itself, or should not be, and it is beyond dispute that the upheaval entailed is in itself a real disincentive. The moral is that manufacturers should as a matter of course sell (or offer to sell) more than a machine—the software should go with it. But it is by now quite clear that the software as sold commercially is often outrageously expensive. If manufacturers want to keep their order books full, they will take steps to see that the machines they sell do actually perform as advertised. Such a goal could bring quite a different structure for the computer industry. As things are, manufacturers have assumed that their profitability is best ensured by apeing the motor car industry and setting up production lines for turning out replicas of more or less identical machines. Although there is undoubted scope for arrangements under which computer complexes can be assembled from smaller parts so as to provide each potential customer with a custom-assembled complex, this is only a beginning. Might it not be more profitable for the manufacturers to find some way of standardizing on software even if this meant that the machinery had to be more sensitively adapted to the customer's needs.

Compromise Without Solution

EVERYBODY knows by now that an arbitration tribunal inquiring into a pay dispute can usually be relied on to split the difference between the two opposing sides. This, predictably enough, is what the public inquiry into the salaries of scientists in the British civil service has done (see page 594). Those who had been offered nothing are now to get an extra five per cent. Those who had been offered something, for practical purposes about ten per cent, will have to be satisfied with that. With masterly empiricism, the tribunal says that the award "seeks to apply the principle of fair comparison and pay research taking into account the special features of a scientific career in the civil service and the fact that the pay of the scientific grades has been determined hitherto by horizontal relativity" without saying why it considers that the arguments put forward by the Civil Service Department in the past few months were invalid, as the Institution of Professional Civil Servants has been claiming. Indeed, the tribunal has the gall to say that "the award should in no way be taken to prejudice future decisions about the unified grading of the scientific and other classes". In other words, the tribunal has casually discarded one of the principles used in previous inquiries like this but has neglected to suggest a replacement. Nobody can be blamed for reacting vigorously to this development. The Civil Service Department will have only itself to blame if the Institution of Professional Civil Servants now becomes a constant thorn in its flesh. Unhappily, even the institution is a long way from recognizing that the time has come to take a much more radical view of the framework within which professional scientists should be employed by the British government.

The most obvious difficulty is that the scientific civil service has become a genteel blind alley for a great many of those who work in it. This is the point that the Fulton commission made. In practice, it has been exceedingly difficult for civil servants who begin as scientists to become, in the course of time, administrators of a more conventional kind. A part of the reason, no doubt, is that many working scientists in the civil service prefer to stick to science—not all of them complain. But it is beyond dispute that for a great many people who would normally expect in other walks of life, in industry for example, to move sideways from research into administration, the present rules are cramping. It is true that the Civil Service Department has been looking for recipes for transforming the civil service along lines that might have been suggested by the Fulton commission and that, ironically, discussions on these have been in part held up by the pay dispute. In the meantime, however, it is plain that the very large proportions of people who have accumulated in the higher grades of the scientific civil service are a measure of the extent to which there are few ways out at the top.

What is to be done? The first thing to recognize is that to the extent that many professional scientists in the civil service value their professional connexions as much as their status in the hierarchy, there is a strong case for hoping that it should be made comparatively easy for them to leave the civil service for other kinds of work. In other words, there should be much easier machinery by means of which scientists in the civil service could

contract out of the arrangements which are conventional. In particular, it should be much easier for them to take their pensions with them if they are so inclined. The simplest way of arranging this would be to arrange that the Civil Service Department would convert into cash or its equivalent the accumulated pension rights of somebody wishing to leave. To be sure, this might often mean that the civil service would lose many men of promise and attainment, but then the question arises what in any case is the civil service for? Is it really meant to be an organization primarily concerned with its own perpetuation? Or should it be more eager than it is to help spread its skill about the country? These and related questions are the ones which the Institution of Professional Civil Servants should now be asking. It would be a great misfortune if they become entirely lost in detailed quibbles about the most equitable way of comparing dissimilar kinds of careers.

100 Years Ago



IN his last weekly return, the Registrar-General refers to the westward advance of Asiatic cholera as investing with more than ordinary interest Dr. Frankland's usual monthly report upon the quality of the metropolitan water supply. The water supplied by the New River and Kent Companies is again reported freest from organic impurity, that of the East London and Chelsea Companies the most impure. With reference to the advantage which would be derived from a general application of Dr. Clark's softening process to the London water supply, the Registrar-General adduces the following valuable facts, which have been communicated by Mr. Robert Rawlinson, C.B., C.E.:—"The average daily water supply to the metropolis was 111,292,104 gallons in June, and 112,107,697 gallons in July. Now in each million gallons of these waters there is about one ton of bi-carbonate of lime, or 111½ tons in June and 112 in July. About two-thirds of this weight of lime or chalk would be removed by Dr. Clark's softening process—that is, in June 74 tons, and in July about 75 tons. In each year about 25,000 tons of useless lime would be removed from the metropolitan waters by the simple and easy process now in use at Canterbury." The Registrar adds: "This riddance of the foreign matter, which deprives water of some of its cleansing properties, is in itself an advantage; but, besides this, the fine precipitate of chalk carries down with it suspended impurities and probably frees it from choleraic and other contagions. It is a most effective filtration." It is a comfort to know that the working classes are beginning to feel their strength. When that is put forward it is certain to be in the direction of sanitary and educational measures. The Registrar-General appends to his report a concise sketch of the steps proper to be taken in view of the threatened epidemic.

OLD WORLD

SCIENTISTS' PAY

Splitting the Difference

THE announcement last week of the award of the Civil Service Arbitration Tribunal to scientists employed in government laboratories brings to an end the first round of the dispute between the Institution of Professional Civil Servants and the Civil Service Department that has gone on since early in June. The IPCS, however, is far from satisfied with the result and is already preparing a campaign to change the whole process of pay determination for government scientists.

The IPCS, after asking for an overall increase of 14.1 per cent for members of the scientific classes, was offered 2.5 per cent by the Civil Service Department—with no increase for the higher grades. The arbitration tribunal awarded 5 per cent to the higher classes not offered any increase originally but was more open-handed with the Scientific Assistant classes. Otherwise the tribunal mostly confirmed the original Civil Service Department offer, details of which were given in *Nature*, **232**, 512; 1971. The Senior Scientific Assistant class was awarded a 5 per cent increase compared with the 10 to 15 per cent asked for and the 2.5 to 3 per cent offered. The Scientific Assistant class received the largest increase with the minimum of the scale being increased from £490 to £550 and the maximum from £1,253 to £1,395—very near to that demanded by the IPCS. The total award amounts to a 7.5 per cent overall increase and will cost the government nearly £2.5 million annually.

The Civil Service Department has cause to be pleased with the award as Mr Mustill, QC, the tribunal chairman, and his fellow members, state that "the award seeks to apply the principle of fair comparison and pay research that also takes into account the special features of a scientific career in the Civil Service".

The IPCS has argued vehemently against this principle (see *Nature*, **232**, 512; 1971) and believes that the pay of the scientist should be based solely on internal relativities. Mr William McCall, the general secretary of the IPCS, considers the award to be thoroughly unsatisfactory and he said last week "(the award) has attempted to produce a compromise between internal relativities and pay research. What it has achieved in fact is a complete mess. The award resolves none of the basic issues involved in the arguments to the tribunal. Indeed, the award marks the beginning of a dispute, not the end of

it. It is plain from the statements of cases submitted to the arbitration tribunal that the salaries indicated by internal relativities cannot be reconciled with pay research information".

The IPCS will face an uphill task in its campaign to determine scientists' salaries in the future by some means other than pay research. Most other salaries within the Civil Service are directly or indirectly determined by pay research, and it will need a very strong case to place the scientific classes outside this scheme. The IPCS is confident, however, that this can be done, and at the moment is pressing for a joint review body of the Civil Service and IPCS to be set up to decide how scientific salaries are to be determined in future. As yet there is no reaction from the government to this suggestion, but it does seem likely that round two is about to start.

ELECTRONICS

Microcircuit Trouble

THE British microcircuit industry is in decline as the world demand for integrated circuits continues to dwindle and overproduction increases. Companies are now faced with the extraordinary situation in which integrated circuits are being sold in the United States for less than the estimated cost of the raw materials and at only slightly higher prices in Britain. Manufacturers such as GEC-Marconi seem to be resigned to cutting production and closing factories.

What has caused such a state of affairs in which an American company, Texas Instruments, can now sell for £0.05 an integrated circuit that would have cost £3.00 in 1967? The first thing to say is that manufacturers of integrated circuits throughout the world are experiencing similar difficulties. Overproduction in the past few years has been the inevitable result of a rapid increase in production efficiency—better crystal growing techniques and a smaller rejection rate for completed circuits, for example—coupled with a reduction in the rate of increase of world demand. Companies that expanded their manufacturing facilities a few years ago, and then gritted their teeth while prices tumbled, are now obliged to prune manufacturing capacity thus losing skilled workers and running the risk of falling behind technologically. Those companies that can resist this inexorable process will presumably reap the benefits eventually.

During the past few months GEC-Marconi has announced the impending closure of its integrated circuit factories at Witham and Glenrothes after sustaining a loss of £1 million on these activities in 1970. Mullard lost £1.4 million

on its microcircuit sales last year and Texas Instruments has made several hundred people redundant at its factory in Bedford. The state of the British market during the past few years is shown in Table 1; price reductions and the small growth in demand have both contributed to the relatively meagre increase during 1970.

Table 1. Annual Sales of Microcircuits in Britain

1966	£2.6 million
1967	£4.8 million
1968	£7.8 million
1969	£15.4 million
1970	£17.0 million

About half of the British integrated circuit requirements in 1970 were imported and 43 per cent of these were supplied duty free from the Free Trade Area and from Singapore (where they are chiefly manufactured by American companies). Most of the other imports were of integrated circuits manufactured in the United States and packaged in cheap labour areas such as Taiwan, Korea and Portugal; these are still relatively inexpensive in Britain in spite of the 20 per cent tariff.

The total British market is much smaller than the domestic market in the United States (about £180 million in 1970) and fluctuations in the American market obviously have serious repercussions in Britain as American manufacturers try to keep production lines going by continuing to sell at a loss. Some Members of Parliament have indeed suggested that integrated circuits are being dumped in Britain and have called for additional tariffs or import quotas, but there is no doubt that dumping, in the strict sense of the word, cannot be taking place when the same integrated circuits are being sold more cheaply in the United States.

ENGINEERING

More Grades Invented

THE Privy Council has now approved the request by the Council of Engineering Institution to set up an Engineers' Registration Board (ERB) providing a composite register of Chartered Engineers, Technician Engineers and Engineering Technicians. The ERB will comprise institutions and societies that are involved in education and training of their members in one of the above categories.

The aim of the ERB, according to the Council of Engineering Institutions, is to rationalize the qualifications of engineers and to give the engineer an identity. The three classes of membership suggested will carry the letters C.Eng., T.Eng.(CEI), and Tech.(CEI),

and the qualifications for membership are a degree or equivalent in engineering for the C.Eng., a Higher National Certificate or City and Guilds Full Technological Certificate for a T.Eng. (CEI) and an Ordinary National Certificate or a City and Guilds Part II Final Technicians Certificate for a Tech.(CEI). Membership also requires appropriate training and experience in all categories.

The C.Eng. qualification is not new and was first instituted when the Council of Engineering Institutions was formed in 1965. The two new classes now instituted will, it is hoped, supersede 50 or 60 titles that have been in use for many years all with different qualification standards. Whereas the Council of Engineering Institutions does not expect all engineers to apply for membership immediately, a spokesman said this week that the eventual goal is to have the presently instituted qualifications accepted as the standard by all British engineers.

Engineers can be nominated to the register by one of the fifteen constituent members of the ERB. There will be no fee directly paid to the board by the engineer—his membership of the parent institution will be sufficient to qualify for membership. The member institutions are enthusiastic about the setting up of the Engineers' Registration Board, but as yet they have not tended to approach their corporate members to ask their views. There seems to be no reason why members should not share this view.

SCIENCE MUSEUMS

Summer Boom in London

IN the summer months, attendance at the London museums reaches its annual peak and the Science Museum and the Natural History Museum—the British Museum (Natural History) as it calls itself—is sharing in the popularity. The Science Museum has had more than 2,000,000 visitors a year since 1968 and the Natural History Museum has some 1,500,000 visitors a year—a figure that is increasing at 10 per cent a year.

But who comes to the museums, and what do they offer? There are no detailed statistics, although a survey that would establish this has been suggested at the Central Office of Information—but both museums know they have to cater for large numbers of children. The Natural History Museum has it down to a considerable art, with a special Children's Centre open on Saturdays during term and six days a week during school holidays. This helps children to find their way constructively around the museum by providing them with "Nature Trails" and encouraging those showing a

genuine interest to join the Natural History Club which meets on Saturdays, when a certain amount of field work is done. There are close links with schools and teachers, and school parties are frequent. From a small random survey last winter, the Natural History Museum has some idea of the age distribution of its visitors—65 per cent of them are under thirty, over half of those are under twenty, and the survey excluded school parties, which provided just under 120,000 visitors in 1970.

The Science Museum has no such figures, but it thinks that one third of its visitors are children and that an appreciable proportion of them come time and again. Arrangements for seriously interested children are meagre, although the museum does run an increasingly comprehensive and varied set of lectures and tours. There is no equivalent of the "Nature Trails"—an experiment with a similar scheme is said to have turned out to be administratively and technically unmanageable. There is, however, some contact with schools and the special Children's Gallery, transferred to a larger location in the basement some two years ago, is as popular as ever. The transfer has allowed for several new exhibits to be included together with the old favourites.

Since 1963, the work of modernizing the exhibitions has progressed gallery by gallery, for the museum is aware that even if new exhibits are continually installed, the design of a gallery dates over the years. A spokesman said that plans are also afoot to redesign the mathematics gallery to include information on computing—a feature that has been sadly neglected to date—with the possibility of a working computer terminal. A section on production engineering is also planned, and both schemes should increase the topicality of the museum.

Although the museum itself makes no complaint, the sum available for new acquisitions is only £8,000 a year, a frighteningly small amount of money if the museum is expected to keep any sort of pace with scientific and technological developments. In spite of the attraction for children in the many working models, the museum does not consider itself primarily for children. They feel that their research facilities are an important part of their work, and that the main aim of the public galleries is to arouse people's curiosity, and possibly stimulate them to a deeper interest.

The Natural History Museum fills rather a different role from the Science Museum. Despite its large public halls, its purposes lie rather in the fields of active research, which the new wing, and the new building at Tring, at present under construction, will help to further. Established in 1881 on a taxo-

nomical basis, the museum is now questioning the validity of this as current research shows more and more that animals and organisms should be considered in their environments, and a spokesman said there may be changes in the future to base the layouts on ecological principles. Before this can be fully accomplished more exhibition space will be needed.

Nevertheless the main purpose of the museum is always likely to be one of research, partly because of the impossibility of displaying more than a small percentage of the natural world—for example the museum owns some 250,000 mineral specimens alone, of which only 4 per cent are on display—and partly because there is much still to be made known about the natural world. After all, although there are some 750,000 known species of insects, it is estimated that there are between two and four times as many yet to be identified.

CONSERVATION

Just for the Birds

THE Royal Society for the Protection of Birds has recently established a 335 acre reserve in the Dale of Cottascarth on the mainland of Orkney. The society, which manages more than forty other bird sanctuaries throughout the British Isles, chose this northern site because there are a number of typical habitats of the birds that breed in Orkney within its boundaries. These reserves are an attempt to maintain the natural integrity of wildlife communities and those that have been in existence for some time have been very successful in this purpose. Access is restricted to members of the society, although this year saw the well established reserves open their doors a fraction to let in small numbers of the interested public. The doors are unlikely to open much wider, and understandably so for the birds' sake.

With the ever-increasing popularity of Orkney with holiday-makers, the establishment of the Cottascarth reserve is wise and timely. The Dale is, in fact, scheduled by the Nature Conservancy as a site of special botanic interest.



Short-eared owl with young.

NEW WORLD

Drug Office Wins Friends, Disarms Enemies

by our Washington Correspondent

ONLY two short months have now gone by since President Nixon announced to Congress and the nation his war against drug abuse, and already two Congressional committees have concluded hearings on legislation designed to put into effect his package deal setting up a Special Action Office for Drug Abuse Control, with additional funding of \$155 million for the control of drugs and the rehabilitation of addicts. Both committees—the House Subcommittee on Public Health and Environment and the Senate Subcommittee on Intergovernmental Relations—are expected to report to Congress soon after the recess, and it seems from the evidence submitted to the committees that the President's proposals should emerge substantially unchanged. The programme has met with approval even from the agencies whose work will be subordinated to the new special action office.

Chief among the tasks of the proposed action office is to coordinate the federal programmes for drug control and rehabilitation, at present spread over nine agencies of the Federal Government. To accomplish this task, the Special Action Office will have at its disposal the effective and overtly simple expedient of complete budgetary control over all federal drug abuse programmes. But creation of the special action office will also underline a marked shift in the government's attitude to drug control, for in the past it has relied heavily on the courts to deter potential addicts from taking drugs, often at the expense of seeming to neglect the other side of the coin—the rehabilitation of existing addicts. Only the full realization of the extent of heroin addiction among troops in Vietnam which came to light about a year ago, and the potential impact of thousands of addicts returning to the United States from Asia has led to the tardy adoption of a crash programme aimed at rehabilitation.

While the new emphasis away from severe court action brought against drug users and towards education and rehabilitation has been widely applauded, there are still many doubts surrounding the way in which the new special action office will function. One such doubt is whether the director of the office will be able to exercise complete control over the budgets of the agencies in his jurisdiction without at the same time running up against resentment within the agencies themselves at their loss of effective power. As James W.

Symington, a member of the Subcommittee on Public Health and Environment, said to Dr Jerome H. Jaffe, Nixon's nominee to head the action office, "in every one of those agencies there is a fellow that has the responsibility right now to see how much they can afford, where it ought to go, and how he exercises that responsibility depends on the multiplicity of contacts that agency has in the states and localities. But you are going to understand all of that for every agency, put it together and then explain to them how to handle it. Congratulations and best wishes".

In many respects, the appointment of Dr Jaffe to the post of director has circumvented a few of those fears, for Jaffe is respected by all the agencies with which he will be dealing. In particular, he has served for several years on the board of the National Institute of Mental Health, whose Division of Narcotic Addiction and Drug Abuse will consume some 70 per cent of the Special Action Office's funds. During that time, Jaffe has built up a strong working relationship with the NIMH staff which will stand him in good stead for regulating their budgets. One official of the NIMH said last week that he regards the special action office as a powerful ally to fight for funds and that there should be no problem in the transfer of power from the NIMH to its drug overlord.

How Jaffe visualizes the work of the special action office became clear during his second appearance before the subcommittee earlier this month. He explained that the office would not seek to meddle closely in the work of the various agencies, and that it would take over the running of a particular programme only as a last resort. The office is therefore intended to be an overlord, working chiefly by informal contact and discussion with the members of the agencies under its control, acting as their mouthpiece in Congressional hearings, and using its powers to seek appropriations for drug abuse control and to distribute federal monies.

Applications for funding for new projects will be channelled through the office, and Jaffe will be concerned especially to see that there is no duplication of effort among federal agencies. The arrangement also has the potential advantage that it may help to speed up the adoption and funding for new projects, for they will no longer be buried within the bureaucratic layers of each individual federal agency.

If the house subcommittee on Public Health and Environment does alter the Administration's legislation, it seems likely that the powers of the proposed special action office will be widened rather than cut down, for there has been some disquiet that the Department of Defense is not included among the Federal agencies controlled by the office. Jaffe, however, has said that there would be no particular advantage in his having budgetary control over the Defense Department's drug abuse programmes, because any necessary coordination can be achieved by informal discussion.

One aspect of the drug problem in which the attitude of the Department of Defense is particularly important, however, is to establish the terms under which a member of the armed services is given a dishonourable discharge. In the past, services personnel have been given a dishonourable discharge for drug addiction, and this has prevented them from receiving treatment in the clinics run by the Veterans Administration.

There is also the problem of administering tests on servicemen for the presence of opiates in the urine, prior to discharge. It has recently been found from such tests that 5.4 per cent of American servicemen in Vietnam had used heroin in the two days before the test was given. The test, which was given on a random basis, almost certainly provided an underestimate of the number of servicemen taking hard drugs, and Jaffe himself believes that the true figure is probably nearer 10 per cent. It therefore seems likely that the subcommittee will add the Defense Department to the legislation in the hope that the Special Action Office will be able to coordinate not only the testing of American servicemen for the presence of hard drugs, but also the treatment of addicts in the armed services.

If the Special Action Office is set up, it will have an initial lifespan of three years, when its operations will be reviewed by the President. There is little in either the proposed legislation or in the steadily increasing figures for drug addiction—it is estimated that there are now about 250,000 heroin addicts in the United States, and probably 150,000 in New York alone—to suggest that the problem will be any less acute in 1974. But at least a change of direction in favour of rehabilitation is a comforting sign of the Administration's intentions.

Ailing Health Services

by our Washington Correspondent

A BATTLE is developing between Congress and the Administration over plans to transfer a narcotics rehabilitation and clinical research centre at Fort Worth in Texas from the Public Health Service to the Bureau of Prisons. The Administration stands accused of effectively shutting down much-needed facilities for the rehabilitation of addicts at a time when a drive against drug addiction has been labelled a national priority.

Congressional opponents of the plan led by Paul G. Rogers, chairman of the House Subcommittee on Public Health and Environment, seem assured at least of staying the execution for another year. A congressional victory may already be too late, however, to enable Fort Worth to carry on business as usual, for while the execution order has been hanging over the hospital, staff morale has slipped so badly that 135 of the hospital's usual complement of 400 have either left or retired during the past year, leaving the establishment severely undermanned.

Fort Worth is one of only two federally run narcotics rehabilitation and research centres left in the United States and, like its sister institution at Lexington in Kentucky, it is administered by the Public Health Service. Between them, the two hospitals cater for about 2,000 narcotics addicts each year committed by the District Attorneys' offices under the Narcotics Rehabilitation Act of 1966. Patients are treated at the hospital for a period of about six months and are then sent to an after-care centre, where they are prepared for return to normal life. In Fort Worth alone, 941 narcotics patients were treated last year.

The thrust of the Administration's policy is to rely less on large in-patient hospitals than on community-based centres. The rationale underlying this approach is that there is more likelihood of success when a patient is treated within his local community, because sending him away to a relatively isolated in-patient hospital causes undue emotional upheaval.

To this end, the National Institute of Mental Health, the agency in charge of the programme, has been setting up community-based treatment centres to cater for patients who would otherwise have gone to Fort Worth, and the intention was to transfer the Fort Worth hospital to the Bureau of Prisons on October 4 for use as a medium security facility for treatment of geriatric, alcoholic and drug addicted prisoners. Opponents of this plan have, however, criticized the Administration for providing inadequate facilities in the com-

munity centres to take up the overspill from Fort Worth, and they therefore point out that the plan will simply result in closure of drug rehabilitation community centres to take up the overspill facilities.

Mr Robert Carrick, deputy director of the NIMH Narcotic Addict Rehabilitation Branch, accepts that more community based facilities are needed, and he pointed out last week that "to say that all are geared up for use is untrue", but he nevertheless suggested that the fifteen centres already in operation have a potential capacity greater than needed to take up the patients from Fort Worth. (The centres are located in Florida, New Jersey, Rhode Island, Puerto Rico, West Tennessee, North Virginia, Arizona, California, Minnesota, two in Mississippi—one of which will serve Kansas—New Mexico, Oregon, Texas and Washington, and there are plans for centres in Louisiana and three more in Texas.)

The centres are operated under contract from NIMH, and the Federal Government reimburses local money spent on the treatment of narcotics addicts under the Narcotics Rehabilitation Act of 1966. Local finance is therefore not a great barrier to operation of a community centre.

Increased capacity notwithstanding, if the Fort Worth facility is closed down, there will be areas of the US that will suffer because they have no local drug treatment facilities of their own. For example, during Congressional hearings on the proposal to set up a Special Action Office for Drug Abuse Control (see page 596), Mr Rogers pointed out that St Louis houses some 6,000 narcotics addicts but that it has only eleven in-patient beds. Similarly, New Orleans has about 6,000 addicts and only ten beds.

For the Administration's plans to treat addicts within their local community to have a chance of working, it therefore follows that drug treatment facilities should be extended to every community in which they are needed, otherwise addicts will still have to be sent away for treatment. This argument is already embodied in legislation introduced into Congress by Mr Rogers and which would prevent payment of grants to community mental health centres after June 30, 1972, unless the grant application is accompanied by assurances that the centre will provide drug rehabilitation facilities.

The legislation is, however, being opposed by the Administration on the grounds that drug rehabilitation facilities may not be needed in every community centre, but even a cursory reading of the Rogers Bill should show that there is just such a get-out clause, for the Bill specifically states that the requirement shall not apply if the Secre-

tary for Health, Education and Welfare "determines that there is not sufficient need for such a program in such hospital or other facility". The Administration therefore seems to be opposing legislation which in effect supports its own position.

The Administration's attempt to close Fort Worth is not an isolated action, for it forms part of a concerted attack on the Public Health Service mounted by almost every successive Administration since the war. So far, Congress has managed to frustrate the present Administration's hatchet wielding against the service, and it looks like being successful in its attempt to keep Fort Worth functioning in its present form for at least another year. The House passed a resolution to that effect earlier this month, and there is no reason to expect that the resolution will be turned down by the Senate.

The House resolution was identical to a resolution passed a few days earlier by the Senate, in that it called for adequate funding for all the Public Health Service Hospitals to allow them to maintain their present functions throughout the present financial year, but the House version also referred specifically to the clinical research centres at Lexington and Fort Worth. The objective of the resolution is to grant a stay of execution to all the eight remaining public health service hospitals so that Congress and the Administration may jointly define a role for them.

Set up in 1798 by the Marine Hospital Act, the Public Health Service hospitals, as they later came to be known, were originally intended for the treatment of American seamen and coastguards. By the end of the nineteenth century, they also discharged some responsibilities in the public health and sanitation fields. The Public Health Service grew slowly in the period up to the Second World War, when it was operating twenty-four hospitals with more than 3,000 beds. Only in the post-war period, when demand for the facilities by American seamen injured in the war slackened off, did the Public Health Service begin to fall foul of the Administration.

All but twelve of the hospitals were closed or converted to outpatient clinics by 1955, and the Budget Bureau was already thirsting after the blood of the survivors, recommending, as it has done ever since, that they be closed. In 1965, the Administration recommended conversion of seven of the smaller hospitals to outpatient clinics, while the Office of Science and Technology recommended that the Public Health Service should continue to operate general hospitals, which should be modernized and given adequate funding.

In the event, only two were closed,

and Congress authorized funds for studies leading to modernization of the remaining ten hospitals. No funds for construction have since been requested, however, and two more hospitals were closed in 1969. The latest attempt to get rid of the Public Health Service came in this year's budget, which simply contained no request for funds to operate the hospitals. The idea was to close the eight Public Health Service hospitals and thirty outpatient clinics, and to turn the Public Health Service Beneficiaries over to the Veterans Administration, severe pressure on the VA's already overburdened facilities notwithstanding.

Although Congress has restored the funds to the Public Health Service, to bring the appropriations up to last year's level, the upshot of the Administration's repeated attacks on the system is that the facilities are rapidly becoming outdated and that the whole system has deteriorated through lack of finance. Repeated recommendations that the hospitals be modernized have been largely ignored, staff levels at the establishments have been allowed to fall and repeated threats of closure have done nothing to stimulate the morale of the Public Health Service's 5,000 doctors. But this deterioration of physical facilities has become one of the Administration's strongest weapons. The pattern is clear: by maintaining the funding for the Public Health Service at minimum levels, the facilities are allowed to deteriorate and the deterioration is later used as an argument for closing the hospitals.

As far as Fort Worth is concerned, there is no doubt that the threat of transfer to the Bureau of Prisons has had a deleterious effect on the staff. As one member said last week, of the 135 who have left during the past year, 85 have retired but expressed their willingness to return if the hospital carries on discharging its present functions.

DDT on Mock Trial

by our Washington Correspondent

A FULL-SCALE public inquiry, billed as the final trial to decide whether or not DDT should be banned from all use in the United States, opened last week in Washington. Chemical manufacturers, in alliance with the US Department of Agriculture are appealing against a cancellation order which the Environmental Protection Agency was forced by federal courts to impose on the pesticide in January this year. Ranged against the Department of Agriculture and the representatives of the chemical manufacturers is the Environmental Protection Agency itself and a variety of environmentalist groups headed by the Environmental Defense Fund.

On the face of things, the hearing could condemn DDT as a hazardous chemical only to be used outside the United States in countries where it is still regarded as an essential weapon in the armoury against malaria. On the other hand, the pesticide could be acquitted and freed for use on certain crops in the United States itself. But the issue is, unfortunately, not that simple. A decision against DDT will in effect only prevent the agent from being sold across state boundaries, and the whole history of the in-fighting and delaying tactics that have accompanied moves to ban DDT in the US shows up the federal pesticide regulatory mechanism for the cumbersome and unsatisfactory procedure that it has become.

Opening shots against DDT were fired in November 1969, ironically by the Department of Agriculture, one of the principal protagonists in the fight to defeat the present cancellation order against the chemical. Amid a blast of publicity, President Nixon then announced that the agriculture department had cancelled four of the existing uses of DDT: spraying tobacco crops and shade trees, use around the home and over water. Thirty days later, however, several producers of the pesticide exercised their rights under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) to request that a scientific advisory committee look into the cancellation order, and thereby set in motion the mills of the pesticides regulatory machinery.

Under the provisions of FIFRA, once an appeal has been filed against a cancellation order, companies are allowed to carry on manufacture and sale of the material under examination. After 1969, pesticide manufacturers were allowed to do this for the seven months that it took for the department of agriculture to set up the appeals committee. Four of the appealing companies withdrew their request for an advisory committee, however, and in July 1970, the Lebanon Company requested a public hearing, but this request was also later withdrawn. What usually happens is that an advisory committee has sixty days in which to file its report, an order either confirming or setting aside the cancellation order is made within ninety days of receipt of the report and sixty days are then available for objections to the second order. An objector has the right to insist on a public hearing on the second order, and a final order is then issued within ninety days of the completion of the public hearing. During the whole proceeding the product can still be legally marketed.

The next step in the campaign against DDT was taken by the Environmental Defense Fund, an environmentalist

group, which on October 1, 1969, filed a petition with the Secretary for Agriculture, requesting cancellation and suspension of all uses of DDT. A suspension order would prevent the product from being sold until the whole regulatory process had been completed. This request was, however, ignored for six months, until the Environmental Defense Fund got a court ruling ordering the Secretary for Agriculture to reply, which he duly did, saying that he refused to cancel all uses of DDT. This decision was challenged by the Environmental Defense Fund in the courts.

In the meantime, the pesticides regulatory machinery was taken out of the hands of the Department of Agriculture and placed under the control of the newly-created Environmental Protection Agency, headed by William D. Ruckelshaus. On January 7 this year, a court in Washington DC ordered the EPA to cancel all uses of DDT, and to re-examine the decision not to suspend its use pending a final decision. Ruckelshaus duly cancelled all remaining uses of the chemical on January 15, but after seeking outside advice he decided not to suspend its use. It is that cancellation order which is now being appealed against in the public hearing which opened last week, and which will probably continue into 1972. Nearly two years after the initial moves against DDT, the pesticide is still being legally manufactured and used in the US, chiefly by the Montrose Chemical Corporation and some thirty other companies which buy from Montrose and process DDT for use. Eighty per cent of the DDT manufactured in the United States is exported for use against malaria, however, and this activity is not being contested.

What are the chances that DDT will be acquitted by the public hearing? At present, it is difficult to judge, but protagonists for the chemical will argue that apart from the value of DDT for controlling pests on cotton crops, a decision against the pesticide could have international repercussions which may slow down the eradication of malaria in underdeveloped countries. Already, Dr Marshall Laird, a member of the World Health Organization, has warned the public hearing of this possibility. The hand of the DDT protagonists will also be strengthened by the difficulties in controlling the recent spread of mosquitoes of equine encephalomyelitis in some Southern states. But on the other hand, four government committees which have studied DDT between 1963 and 1969 all recommended phasing out its use, and the Mark commission recommended elimination by December 1971 of all uses of DDT not essential to public health.

NEWS AND VIEWS

Rutherford and Geiger Re-evaluated

ADVANCES in nuclear physics in the past decade have arisen chiefly from developments in instrumentation coupled with the higher energy accelerators that became available in the 1960s. It is more than possible that the suggestion by Dr Peter Rice-Evans on page 625 of this issue of *Nature* will eventually be developed into a technique that will have universal application, although the theoretical predictions have yet to be tested. There are certain to be many Jeremiahs who will deem the idea for a new high energy particle detector as unpractical, but there will be few who will argue that the idea is not worth testing.

Rice-Evans's suggestion is based on the proportional counter concept first developed by Rutherford and Geiger in 1908. The novelty of the present approach is that the proportional counter be used in reverse with the outer cylinder being held at a positive potential with respect to the centre wire. The electrons liberated by the passage of an ionizing particle will thus be accelerated towards the outer cylinder and not towards the centre as in a conventional proportional counter. In this way a pulse with a much faster rise can be expected and the instrument becomes, in principle, a powerful tool that can be used to determine the position of an ionizing particle with a higher degree of precision than is currently possible.

To appreciate the advantages to high energy physics if this detector ever became a practical proposition it only needs to be realized that there is a great deal of current concern that the resolutions currently available with wide plane spark chambers, streamer chambers and multi-wire proportional counters will not be adequate to meet the requirements of the experiments that will be carried out with the new generation of synchrotrons typified by the one being built at Batavia in Illinois, and the 300 GeV machine planned for CERN. The higher energy of the accelerated beam means that the analysed particles will also have a higher energy and will thus suffer smaller deflexions in analysing magnets than particles accelerated in machines of lower energies. There is a need therefore for detectors with good spatial resolution and none of the detectors in current use meets the requirements.

How does the proposed reverse proportional counter supply the necessary high resolution? In such a set up the avalanche produced by an ionizing particle entering the chamber will be outwardly directed, and pulses will only arise in the wire when electrons are liberated near the wire surface in the region of high field necessary for avalanche growth. This is to be compared with a conventional proportional counter where a particle is detected irrespective of where in the chamber the event occurs. The difference emphasizes that the proportional counter was initially designed to maximize the efficiency rather than the spatial resolution as in the present proposed use. The analysis of Rice-Evans shows that a pulse produced by an ion within the critical distance needed to form the avalanche achieves 22 per cent of its maximum height within 10^{10} s of the event occurring. This fast rise is ideally suited for obtaining a fast pulse from a differentiation network to trigger the electronic circuits

that are necessary in high energy physics experiments. Thus to obtain a pulse from the chamber an electron must be formed near the wire surface within the critical avalanche radius. It is here that the crucial question of the efficiency of the proposed device arises. To obtain maximum efficiency the radius of the wire needs to be as large as possible, but large wire radius is incompatible with the good spatial resolution that the detector sets out to achieve in the first place. Thus the ever present problem of achieving a compromise between efficiency and resolution would seem to limit the usefulness of the detector.

Calculations by Rice-Evans show that for a typical chamber the region of interaction is limited to a cylinder about the central wire of radius approximately twice that of the wire itself. The efficiency of producing an electron within this region depends on whether the interaction occurs in the wire or in the gas of the chamber, and the probability for these processes is estimated to differ by an order of magnitude—the interaction in the wire being most likely to produce an electron. Thus, the detector radius is effectively the same as the wire radius, and to achieve both high efficiency and high resolution a matrix of many wires will be needed. Such systems are already in use with multi-wire proportional counters.

It is now time to carry out an experimental study with the reverse proportional counter. The problems to be overcome are certain to be many, but only time will tell whether they are insurmountable. Multi-wire proportional counters were first used in 1956, but have only recently achieved universal usage. If the suggested detector is to be used with the new generation of high energy accelerators, however, it has to be developed at a faster pace. The decisions to be made before starting experimental work are many and involve such fundamentals as the material to be used for the wire, what gas to be used in the chamber—or whether a noble liquid could be used instead. The dimensions of the chamber have also to be detected, but of more fundamental importance is the question of how spurious positive ions might affect the performance. Such ions might cause ionization of the gas in the high field region or they might cause secondary electrons to be freed from the surface of the wire. A possible way to circumvent these problems would be to introduce a vapour to neutralize the spurious gas ions and so quench the discharge.

The possibility of the detector being a viable proposition depends entirely on how the expected one to several thousand signal to noise ratio that will arise from particles forming ions outside the avalanche region can be overcome. For a chamber of the dimensions given by Rice-Evans the ratio is one to 5,000 as only 1.5 per cent of the particles will pass through the avalanche region.

The chances of a successful detector being developed in the face of all these uncertainties must be rather small, but several innovations that have in the past seemed to the scientists of the day to have even less chances of developing into viable entities are now accepted techniques. Such a detector or series of detectors must be built and tested.

Earth Sciences Converge in Moscow

from a Correspondent

At the fifteenth general assembly of the International Union of Geodesy and Geophysics (IUGG) held in Moscow during the first fortnight of August it was clear that at long last, and very belatedly, Earth scientists of various disciplines are beginning to talk each other's language. All interests were catered for at the specialized symposia organized by the various member associations within the union. What follows is a selection of items from some of these symposia; a further report will appear in the forthcoming issue of *Nature Physical Science*.

East Africa, and particularly its rift system, has been the site of major British scientific endeavour for many years and this was evident at the East African Rift symposium where eleven of the contributions were by scientists from the United Kingdom. Eight other countries were also represented and this was the first time so many scientists working in East Africa have gathered together.

Seven speakers dealt with the Afar triangle (the junction of the East African rifts with the Red Sea and Gulf of Aden). The structure of the region was ably reviewed by Mohr (Smithsonian Institution) and the volcanology was discussed by Tazieff (CNRS). On display were two maps of the region, a preliminary Bouguer gravity map by Makris (Hamburg) and a very impressive aeromagnetic map by Hall (Newcastle upon Tyne). Hall's map was coloured in such a way that the quiet zones could be readily distinguished from the regions of large magnetic anomalies similar to those found over the seafloor spreading zones of the Gulf of Aden and the Red Sea. Geologists and geophysicists all agreed that sialic crust must be absent beneath much of this region. This view was confirmed by Jobert (Paris) who described some seismic refraction experiments carried out by dropping bombs in the sea and recording on land in the French territory of Afar and Issas. All his profiles showed the presence of oceanic crust. Only the exact proportion of oceanic to continental crust remains uncertain.

From Afar, the theme of the meeting moved to East Africa. A general review of continental rift zones was given by Milanovsky (Moscow). Knopoff (Los Angeles) then presented evidence which convincingly demonstrated the presence of low seismic velocities beneath the East African rift on the basis of surface wave phase velocities at the Addis Ababa and

Nairobi stations. There then followed two geological contributions on the rift volcanics in Kenya; Williams (Nairobi) reviewed the distribution in space and time of the various volcanic rocks, contrasting those occurring on the rift floor and shoulders, and McCall (Victoria) described a special field-geochemical study of the Silali volcano. McCall took the view that the eastern rift is an "aborted" ocean which has never opened up; the rocks are from primary mantle derived material and the magma chambers probably reached to within a kilometre or so of the rift valley floor. Such a view is consistent with the mapping and interpretation of the positive gravity anomaly along the rift axis by Searle (now at Addis Ababa) to which many contributors made reference.

The afternoon session opened with an account by Logachev (Irkutsk) of Soviet expeditions between 1967 and 1969. Soviet scientists have an impressive collection of geological data and have made more than 250 potassium-argon age determinations for 170 different localities in Kenya, Tanzania and Uganda. On the basis of this work, it is thought the process of rifting can be divided into six stages—23–16; 23.5–12; 10–5; 5–2; 2–0.7; and 0.7–0 million years—and each of these represents definite stages of magmatism and structural development. Rykounov (Moscow) described Soviet microearthquake studies in the southern part of the Gregory rift and the northern part of the western rift. In northern Tanzania, he and his colleagues have obtained a crustal structure consisting of 18 km with a P-wave velocity of 5.8 km s⁻¹ underlain by 19 km with a P-wave velocity of 6.5 km s⁻¹.

Fairhead and Girdler (Newcastle upon Tyne) discussed the relocation of epicentres using the joint epicentre technique. Their plots of epicentres on new fault maps prepared by Baker showed a remarkable correlation between earthquakes and recent geological features. They have also calculated travel time corrections for fifteen seismic recording stations throughout Africa and have tentatively mapped regions in which P-waves are slowing down beneath the rift system north of the equator. From a study of all available fault plane solutions they suggest that this region is associated with a W.N.W.–E.S.E. stress pattern and with a consequent thinning of the lithosphere.

A notable feature of this meeting on the East African Rift was a consensus of opinion that there must be some

separation of the lithospheric plates beneath Afar but none beneath East Africa. It seems that the lithosphere in East Africa has thinned but not broken apart.

At first sight, the choice of ocean floor spreading as a major topic for discussion in an international symposium seems somewhat outmoded, for the major impact of this all-embracing theory came 3–4 years ago and is accepted, in varying degrees, by most Earth scientists. Several speakers dealt with the internal processes that cause the seafloor to spread and the plates to move. Models presented by Turcotte (Cornell) and McKenzie (Cambridge) involved the evaluation of solid state thermal convection as the major driving mechanism. Both suggested that the convective process extends deeper into the Earth than the low velocity layer and that total mantle convection could not be ruled out.

Although much of the ocean floor has been investigated by geophysical methods (one eminent US oceanographic geophysicist bemoaned the fact that there was little of the ocean floor left to study), a group of speakers presented the results of regional studies in localized areas; for example, Talwanie and Pitman (Lamont-Doherty Observatory) discussed the Norwegian-Greenland Sea, Krause and Shilling (Rhode Island) gave an account of the Azores area and Barker (Birmingham) described the active spreading centre in the Scotia Sea.

Although there is now a vast amount of data on the physics, chemistry and shape of the Moon, the "vulcanists" and "impactors" at the symposium on this subject seem to be as widely divided as ever. Those selenologists who proposed an internal origin for the Moon's physical phenomena seemed to be dominant, if only because they were thicker on the ground than their opponents.

Because it is to be replaced by the Inter-Union Commission for Geodynamics, the Upper Mantle Project made its last international appearance in a 4½ day symposium.

The lasting impression of the IUGG assembly is that the next decade should be one of great stimulation and interest as continental geological and geophysical data are reappraised in the light of the seafloor-spreading-plate tectonics concept. It is to be hoped that the newly formed Inter-Union Commission for Geodynamics will make a major contribution by guiding these studies.

ADENOVIRUSES

Endonuclease Activity

from our Cell Biology Correspondent

REPORTS of the discovery of this or that enzyme in particles of one or another of the enveloped viruses are these days mundane events, unless, of course, the enzyme happens to be as unusual as reverse transcriptase. By contrast the discovery of enzyme activities associated with the virions of encapsidated viruses is still a comparatively rare event. One such is Burlingham, Doerfler, Pettersson and Philipson's report (*J. Mol. Biol.*, **60**, 45; 1971) that the endonuclease activity which appears in cells infected with adenovirus 2 or 12 and is also associated with purified virions seems to reside in the penton base subunits of the capsids of these viruses.

This new endonuclease activity first came to light when Burlingham and Doerfler (*J. Virol.*, **7**, 707; 1971) were studying the replication of adenovirus 2 and 12 DNA in KB cells and a variety of other permissive and nonpermissive hosts. Burlingham and Doerfler were able to identify three classes of viral DNA in infected cells; molecules which cosediment with DNA extracted from virions and appear to be uncoated parental viral genomes; molecules of adenovirus DNA which are considerably smaller than intact genomes; and finally DNA molecules which are much larger than genomes and may well be parts of the host genome carrying integrated adenovirus DNA. Kinetic studies of the appearance of these three classes of DNA during the course of adenovirus replication lead Burlingham and Doerfler to the conclusion that many adenovirus genomes are cleaved by an endonuclease which appears in the cells after infection and that the cleaved molecules may well be those which are integrated into the host genome. Furthermore, although the experimental details have yet to be published, Burlingham and Doerfler have apparently obtained evidence that the endonuclease is not only a virus-coded protein but also remains associated with pure virions.

Collaborating with Pettersson and Philipson, who with their colleagues at Uppsala have extensively analysed the polypeptides in the three structural units of adenovirus capsids (the hexons, penton bases and penton fibres), Burlingham and Doerfler have tested the capsid subunits for endonuclease activity. Only the penton bases, purified from infected KB cells rather than from pure virions for technical reasons, have this activity, and both selective digestion of the penton bases with trypsin and antiserum to penton base inactivates it. The endonuclease activity is specific for DNA; its activity is twenty-fold greater

with native DNA than with denatured DNA as substrate and it cleaves both strands of double-stranded DNA by some "double-hit" mechanism. Moreover, the homogeneity of the cleavage products produced by the endonuclease with adenovirus DNA as substrate suggests it attacks specific sites which are probably rich in G.C base pairs because poly dG.poly dC is a strong competitive inhibitor whereas poly d(AT) has little effect, and actinomycin D partially inhibits the activity. Assuming that penton bases in capsids have the same activity as those extractable from infected cells, Doerfler and Burlingham have provided an interesting example of a viral protein which as well as forming part of a capsid also plays a part in the replication of the viral genome.

Also in the current issue of the *Journal of Molecular Biology* (**60**, 185; 1971) Landgraf-Leurs and Green report their various attempts to exploit the differential binding of synthetic RNAs by the DNA of adenoviruses 2, 7 and 12 for the separation of the complementary

strands of these genomes. Apparently poly(U.G) molecules with judiciously selected ratios of U and G bind to different extents to the two strands of these three genomes and separation of complementary strands is possible. To prove their point Landgraf-Leurs and Green report the separation on a preparative scale of the strands of adenovirus 2 DNA and self annealing and hybridization experiments show there is no detectable cross contamination.

PHOTOMORPHOGENESIS

Ach Implicated Again

Gressel, Strausbauch and Galun from the Weizmann Institute provide on page 648 of this issue of *Nature* what may be the first evidence that acetylcholine has a part to play not only in plant photoresponses initiated by red light, but also where blue light provides the stimulus. They have shown that sporulation in the fungus *Trichoderma viride*, which is induced normally

Drug Resistance and Ribosomal RNA

ANTIBIOTICS such as streptomycin, spectinomycin, erythromycin and fusidic acid all impair to greater or lesser extents the faithful translation of messenger RNAs in *Escherichia coli*. Resistant mutants can, however, be isolated and, as the pioneering work of Nomura and his associates first established, the loci controlling such resistance specify structural proteins of ribosomes or, in the case of fusidic acid resistance, as other groups have shown, the locus specifying the translocation factor G. But it would be wrong to run away with the idea that mutations leading to resistance to antibiotics which affect translation are confined to genes that specify proteins which are part of the translation machinery. For Helser, Davies and Dahlberg report in next Wednesday's *Nature New Biology* that a mutation which results in resistance to the drug kasugamycin causes a failure in the methylation of 16S ribosomal RNA.

Sparling has shown that kasugamycin interacts with the 30S ribosomal subunit of wild type *E. coli* ribosomes. With the expertise required for the reconstruction of ribosomes from their constituent RNAs and proteins, which has been chiefly developed by Nomura, Helser and his colleagues, at hand at the University of Wisconsin, set about reconstructing 30S ribosomes with proteins and RNAs from either wild type of kasugamycin resistant cells. They quickly discovered that resistance to this drug is not the consequence of a mutated 30S ribosomal protein. They therefore compared fingerprints of the

16S ribosomal RNAs of resistant and sensitive strains, and sure enough found a difference. An oligonucleotide sequence dimethyl-AACCUG present in digests of wild type 16S RNA is replaced by the unmethylated oligonucleotide AACCUG in digests of the 16S RNA from resistant strains. Using RNAs labelled in their methyl groups they confirmed this difference, and further reconstruction experiments proved that resistance or sensitivity to kasugamycin depends on the source of the 16S ribosomal RNA and not on the source of ribosomal proteins.

Apparently therefore the mutation to kasugamycin resistance results in the failure to dimethylate an adenine residue near the 3' terminus of the 16S ribosomal RNA. Helser *et al.* favour the idea that the mutated locus specifies a methylase responsible for adding the two methyl groups to this adenine residue, although alternative explanations of their findings have not been rigorously excluded. As they note, if the kasugamycin resistance locus does specify a methylase probably more than one species of methylase is involved in methylating ribosomal RNA. For even in the kasugamycin resistant strains the 23S RNA of the large ribosomal subunit is methylated normally.

In short, Helser *et al.* have proved that the function of ribosomes can be altered by mutations which affect ribosomal RNA and they have provided circumstantial evidence suggesting that more than one methylase may be involved in the specific methylation of ribosomal RNA.

by small amounts of blue light, can be initiated by treating the fungus with a mixture of acetylcholine and the acetylcholinesterase inhibitor, eserine.

That acetylcholine, known chiefly for its role in the transmission of nerve impulses in animals, may be active in plant photomorphogenesis is a recent and, on the face of things, bizarre idea. But acetylcholine is known from animal studies to mediate certain processes involving changes in membrane permeability, and it is just these sorts of changes which many plant physiologists believe to be the basis of some aspects of photomorphogenesis.

M. J. Jaffe showed last year that mung bean seedlings not only contain appreciable quantities of a substance which the results of a series of tests suggested strongly to be acetylcholine, but also that the levels of acetylcholine in the plant changed characteristically after exposure to red light. Furthermore, acetylcholine, when given to dark grown seedlings, mimicked the effect of red light in certain photomorphogenic responses. One of these responses was the odd electrical phenomenon discovered by Tanada, where mung bean root tips cling to a negatively charged wet glass surface in red light and release in far-red light (see *Nature New Biology*, **230**, 257; 1971). The implication is that acetylcholine is involved in electrical changes in the plant which presumably follow the photoconversion of phytochrome.

The results that Gressel *et al.* have obtained for *Trichoderma* are not so satisfying as Jaffe's for mung bean, perhaps, but they do imply that there may be a fundamental similarity underlying some red and blue light induced photoresponses. *Trichoderma* sporulates in culture after exposure to blue light but not in darkness. Acetylcholine alone cannot replace blue light in this system, but Gressel *et al.* were able to induce sporulation in the dark using acetylcholine in combination with eserine. The relative amounts needed were specific; sporulation equivalent to 95 per cent of the control value was obtained when 10×10^{-5} M acetylcholine was used with 5×10^{-6} M eserine. Curare, an acetylcholine antagonist, suppressed sporulation in the light control material.

Gressel and his colleagues, however, have not indicated very clearly how they think their data applies to the natural regulation of photomorphogenesis. Is, for example, acetylcholine found naturally in *Trichoderma*, and if so what changes take place after exposure to blue or red light? Does addition of acetylcholine and eserine to the fungus really step round the mechanism controlling sporulation or merely provide the operating factors for altering membrane permeability? The authors tentatively suggest that should acetyl-

choline prove common to the mechanism of both blue and red light photomorphogenesis, their modes of action will have to be thought out anew. Very true; but these latest results say less about this prospect than raise questions about the basic nature of the changes which lead to sporulation in blue light sensitive fungi.

RHIPIDISTIA

Links with Tetrapods

from our Vertebrate Palaeontology Correspondent

IN the study of the course of vertebrate evolution, one of the most important groups of animals are the extinct rhipidistian fishes. Rhipidistia first appear in the fossil record in the early Devonian and persist into the Permian and it is generally agreed that this group gave rise to all the tetrapods—amphibia, reptiles, birds and mammals. Important information on the anatomy of these forms is contained in two recent articles written jointly by S. M. Andrews and T. W. Westoll (*Trans. Roy. Soc. Edinb.*, **68**, Nos. 9 and 12; 1971).

As would be expected vertebrate palaeontologists have devoted considerable time to the Rhipidistia, but detailed study has been largely confined to de-

scription of the skull and speculations about their relationships have been based almost exclusively on cranial anatomy. This is very strikingly the case with *Eusthenopteron*. This fish is known chiefly from the early Upper Devonian of Canada and has been a focus of attention since the early years of this century. The skull is probably better known than that of any other fossil vertebrate, and has been studied particularly by Jarvik who has used the technique of serial grinding originally perfected in Sweden by Stensiö. Although there have been many partial accounts of the rest of the skeleton, a comprehensive, detailed and accurate description of the rest of the skeleton has been lacking, so that the work of Andrews and Westoll on the postcranial skeleton of *Eusthenopteron* is welcome.

In their first article, a description of the paired pectoral fins is accompanied by a detailed analysis of the probable muscle origins and insertions of the fins and shoulder girdle. The resemblance of the proximal part of the fin to the primitive tetrapod limb was already known in general terms; detailed analysis now shows that the range of movements of the early tetrapod humerus is almost identical to that of the first axial element of the fin of *Eusthenopteron*. This part of the rhipidistian fin seems in

Structural Gene of Diphtheria Toxin

IN next Wednesday's *Nature New Biology*, Uchida, Gill and Pappenheimer report an elegant series of experiments which prove that the structural gene for diphtheria toxin is carried by the lysogenic bacteriophage β and some of its close relatives. It has, of course, been known for several years that strains of the diphtheria bacillus *Corynebacterium diphtheriae* which do not make the toxin are rendered toxinogenic when they are lysogenized by bacteriophage β and that the yield of toxin depends on the strain of bacterium and its physiological state. Moreover, the *tox* character of phage β maps at a single locus.

In an attempt to decide between the two alternative explanations of these findings, namely that the *tox* gene specifies the toxin protein or that it regulates the expression of a structural gene for the toxin in the bacterial chromosome, Uchida *et al.* devised an interesting selection procedure which enabled them to isolate a mutant of phage β . Cells of non-toxinogenic strains of *C. diphtheriae* lysogenized by this mutant phage make a protein which cross reacts with anti-toxin antibody but is not toxic and lacks the C terminal sequence of the normal toxin molecule. Clearly, therefore, the *tox* gene specifies the toxin protein itself and the mutation

Uchida *et al.* have isolated is either a deletion or a nonsense mutation which prematurely terminates the synthesis of toxin protein.

These findings lead Uchida *et al.* to speculate about the evolution of the *tox* gene. Diphtheria toxin, the substrate of which is restricted to eukaryotic cells, has no role in the replication of phage β and its relatives; it can, for example, be totally deleted without altering the rate of phage replication or the yield of phage. Neither does the toxin have any apparent role in the life cycle of the diphtheria bacillus growing in culture.

Why then has the gene been retained? Uchida *et al.* suggest that the ability to make toxin must have some survival value for the bacillus, and therefore its lysogenic phages, in its natural habitat—the throats of animals and man—and they point out that widespread immunization with diphtheria toxoid has resulted in the eradication of toxinogenic strains of *C. diphtheriae* from many human populations. As for the origin of the toxin gene, Uchida and his colleagues speculate that because the only known substrate of the toxin is found exclusively in eukaryotic cells, the toxin gene may have once been a eukaryotic gene which the bacilli or their phage picked up and modified to their own advantage.

fact to be a true tetrapod humerus.

Other resemblances to the anatomy of early tetrapods are also confirmed. *Eusthenopteron* has two-headed dorsal ribs, albeit with very little blade, throughout the trunk. Furthermore, the pelvic girdle probably had a ligamentous attachment to the vertebral column in the sacral region. This condition would be the expected forerunner of the tetrapod sacrum with a bone-to-bone articulation of the girdle to one or more pairs of specialized sacral ribs.

The most interesting differences between the postcranial skeleton of *Eusthenopteron* and those of other rhipidistians, described in the second article, concern the structure of the vertebrae. The vertebrae of *Eusthenopteron*, inadequately described before this account, have been cited in the decades since the war as the ideal forerunners of the tetrapod condition. The other rhipidistians, however, have a bewildering variety of vertebral types, and as more becomes known of the condition in very early, pre-Coal Measure tetrapods the orthodox theory of vertebral evolution in early land vertebrates becomes more and more unsatisfactory.

Description of the skeletons of rhipidistians other than *Eusthenopteron* also allows a more rational classification within the group and one is presented by Andrews and Westoll. These two articles are a remarkable achievement and will long remain an essential source of information for students of vertebrate evolution.

CONTINENTAL DRIFT

Initiation of Rifting

from our Structural Biology Correspondent

KNOWLEDGE of the timing of the opening of the Atlantic Ocean and of the mechanism involved is extremely hard to come by. The lack of clear magnetic anomaly patterns in the "quiet" magnetic zone on both sides of the Atlantic floor has required an extrapolation backwards of spreading rates from the well established younger central zone of the Atlantic in order to gain approximate dates for the opening. This method gives a wide range of dates for the commencement of opening from Late Permian onwards, depending on the spreading rate favoured.

Another method which has been fairly successfully used has been to determine two sets of magnetic pole positions from continental rocks to either side of the Atlantic during successive stages of Earth history from the Carboniferous onwards. By rotating one of these sets of poles by the equivalent of the amount required to close the Atlantic, it is possible to see at what stage the two sets of pole positions fail

to coincide, thus giving the first point of Atlantic spreading. Larson and La Fontain (*Earth Planet. Sci. Lett.*, **8**, 341; 1971) found by this method that the Atlantic started to open at the close of the Triassic.

On purely geological reasoning, Hallam (*J. Geol.*, **79**, 129; 1971) suggests that spreading started during Lower to Middle Jurassic at the time of the breakdown to a deeper water environment of the widespread and long-lived carbonate platform of the Mediterranean region. He further suggests that downwarping of the southern North Atlantic borders with associated faulting and volcanic activity during the Upper Triassic indicates a period of rifting occurring some 50 million years before the actual separation of the continents. This model is in good accord with a Rift Valley/Red Sea pattern of oceanic opening.

Further corroboration of Hallam's initial tensional phase before spreading

has recently been supplied by May (*Bull. Geol. Soc. Amer.*, **82**, 1285; 1971). He has found that Late Triassic doleritic dyke swarms in eastern North America, West Africa and north-eastern South America fall into a regular radiating pattern if these continents are restored to their relative pre-drift positions (essentially according to the Dietz *et al.* reconstruction (*Bull. Geol. Soc. Amer.*, **81**, 1915; 1970)). The pattern is roughly convergent on the Blake plateau, the Bahama platform and the western Senegal basin, and individually the dykes bear no particular relationship to the structures of the continental rocks through which they intrude. It thus seems that the stress field responsible for the dyke intrusions was unrelated to continental crust tectonics but rather, as the dolerites themselves are of typical oceanic composition, to activity within the upper mantle.

May is able to show that the intrusion of the dykes was related to a tensional

The Extent of Reverse Transcription

It is hard to believe that the chief role of reverse transcriptase in tumour viruses is something other than the synthesis of a double stranded DNA provirus as Temin has proposed. And Duesberg and Canaani have already reported, as expected, that the reverse transcriptase in Rous sarcoma virus transcribes *in vitro* all the sequences present in the viral RNA genome, albeit as a series of small DNA molecules rather than a single DNA molecule as big as the viral genome. In next Wednesday's *Nature New Biology*, however, Varmus, Levinson and Bishop describe a more detailed analysis of the products of reverse transcription of Rous sarcoma RNA which suggests that at least *in vitro* some sequences of the viral genome are preferentially transcribed into double stranded DNA.

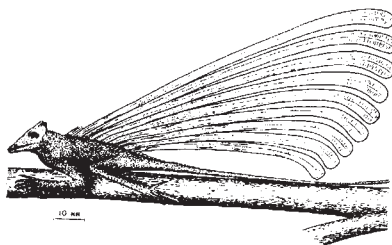
Reverse transcription takes place in two steps; the first is the synthesis of a single DNA strand complementary to the viral RNA genome to yield a RNA.DNA hybrid; the second involves the synthesis of a double stranded DNA using this hybrid as template. When Duesberg and Canaani measured the extent of transcription *in vitro* of Rous sarcoma virus RNA they hybridized the total DNA made, both single and double stranded, to viral RNA.

What Varmus *et al.* have now done is measure the extent of reverse transcription *in vitro* into double stranded DNA. After separating the single stranded from double stranded DNA made by Rous sarcoma virus reverse transcriptase they measured the rates of reassociation, after denaturation, of the double stranded product.

Most of this DNA rapidly reassociated, a small proportion slowly reassociated and some failed to reassociate. By making various assumptions Varmus *et al.* estimated the molecular weight of the rapidly reassociating fraction and what fraction of the total Rous sarcoma genome it corresponds to. They claim that from 85–90 per cent of the double stranded DNA is probably transcribed from about only 5 per cent of the viral genome assuming its molecular weight to be 10×10^6 .

But does the slowly reassociating fraction of the double stranded DNA contain sequences corresponding to the entire viral genome? Varmus *et al.* claim, from measurements of the rate at which it reassociates, that this double stranded DNA corresponds to only about 20 per cent, rather than 100 per cent of the genome. Moreover, they report experiments which they interpret as showing that at least 30 per cent of the DNA sequences found in RNA.DNA hybrids are not transcribed further into detectable amounts of double stranded DNA. They mention, however, unpublished experiments of Martin and his colleagues who apparently also find a major rapidly reassociating fraction and a smaller slowly reassociating fraction of double stranded product but who believe the smaller population of molecules include all the sequences of the complete viral genome. It is perhaps too early to decide which interpretation is correct, but it seems clear that a small part of the viral genome is transcribed *in vitro* into double stranded DNA much more often than into the complete genome.

Triassic Glider



THIS illustration shows a reconstruction of the lower Triassic reptile, *Longisquama insignis*, the remains of which were recovered by A. G. Sharov of the Soviet Academy of Sciences in the river clay deposits at Fergami in the Turkistan Ridge, 60 km from Leninabad (*Priroda*, No. 7, 108; 1971). The spinal appendages in this pseudosuchian were plate-like and were arranged in pairs down the spine. In addition to the skeleton, Sharov also found the scaly cover of the body and extremities.

phase affecting a broad area of crust before the drifting of the continents. He constructed two-dimensional stress trajectories (orthogonal lines representing variation in maximum and minimum stress directions) by drawing lines normal to the dyke trends (minimum stress) and lines parallel to the dyke trends (maximum stress). From these trajectories it is not only possible to determine surfaces of regional tension but also surfaces of regional shear and it was these surfaces that were later utilized to form the spreading axis of the Atlantic. The continental margins of North America north of the Bahamas and of West Africa north of Senegal broadly follow tension lines as does the southern coast of West Africa off Liberia. But from the Ivory Coast west to Nigeria the continental margin follows a direction of shear possibly related to the Romanche fracture zone.

Furthermore, the stress trajectories show a concentration of stress in an area around the Bahamas Ridge—a point not made by May but apparent from his construction. This concentration of stress in the upper mantle resulted in the tensional phase over a wide area of the overlying crust with the consequent dyke intrusion in the Late Triassic. At a later date the continental crust split, possibly initially at the point of stress concentration and subsequently along incipient lines of tension and shear. With the onset of rifting the stress on the continent was relieved and further displacements of the mantle resulted only in the production of new oceanic crust in the rift.

SENSORY PHYSIOLOGY

Olfaction and Taste

from a Correspondent

MOST of the research discussed at the fourth international symposium on olfaction and taste, held at Starnberg, Munich, between August 2 and 4, illustrated the progress which has been made in the understanding of the morphology and electrophysiology of single sensory cells or sensory epithelia, with relatively little emphasis on the central processing of chemosensory information or the influence of chemical stimuli on behaviour.

To elucidate the transduction mechanism by which chemical stimuli induce electrical changes in cells, it is convenient to be able to obtain reasonably large quantities of receptor membrane for analysis of the composition and conformation of the receptor surface. Dr J.-P. Changeux (Institut Pasteur, Paris) has used cell fragments of the electroplaques of the electric eel as a bulk preparation of cholinergic receptors and has been able to extract protein fractions which seem to bind acetylcholine, carbamylcholine and decamethonium.

The ubiquitous *Escherichia coli*, which is also readily available in bulk, has been used by Professor J. Adler (University of Wisconsin) to investigate the molecular basis of chemotaxis. Several species of receptor molecule on the receptor surface of the bacterium interact selectively with one of a group of substances to influence the direction of movement of the organism.

In spite of the difficulty of preparing pure extracts for analysis, sugar binding proteins from the sensory cells have been identified in cow tongue by Dr M. Sato (Kumamoto University) and in rat tongue by Dr K. Hansen (Zoologisches Institut, Heidelberg). In some particularly small scale experiments with blowflies, Dr H. Morita (Kyushu University) has found α -glucosidase activity associated with single contact receptor endings. Much more work will be needed, however, before it is understood how such interactions may be associated with changes in the ionic permeability of sensory cell membranes. Dr U. Thurm (Ruhr University, Bochum) has demonstrated that part of the total potential difference across insect sensory epithelium is dependent on metabolic energy. No further evidence was presented, however, to

Skiddaw Slates and Borrowdale Volcanics

THE rocks of many areas have been greatly faulted and cleaved as a result of Earth movements following their deposition. Over the years much geological work has been carried out in the Lake District, as elsewhere, to unravel the types and ages of Earth movements which have disturbed the country rocks. At the base of the stratigraphical succession of the sedimentary rocks of the Lake District are the Skiddaw Slates, of Ordovician age, on which rest the Borrowdale Volcanic rocks, also considered to be Ordovician. These in turn are succeeded by the Coniston Limestone Group (Ordovician) on which lie the Silurian flags, grits and shales.

The junction between the Skiddaw Slates and the overlying Borrowdale Volcanic rocks has been variously described as conformable, an unconformity, or faulted. A considerable obstacle to a confident opinion on this problem is the rarity of clear sections of the junction, because the surface of the country is widely covered by glacial and other drift deposits.

The Coniston Limestone Group succeeds the Borrowdale Volcanic rocks and it is now widely thought that the junction between these two groups of strata is markedly unconformable.

A thick succession of shales, flagstones, grits and greywacke lie above

the Coniston Limestone Group and these belong to the Silurian System. These beds are succeeded with marked unconformity by the local representatives of the Old Red Sandstone (Devonian) or Carboniferous Limestone Series.

Recent wide scale mapping, involving the collection and plotting of large numbers of dips, strike lines and faults, suggests that the structure is more complicated than previously supposed. In particular, the relationship between the Borrowdale Volcanic rocks and the underlying Skiddaw Slates has been examined in great detail where exposed, but opinion still differs as to whether the junction is conformable, unconformable or faulted and, if the last-named, the nature of the faulting involved. Some workers, indeed, claim to recognize six groups of minor structures in the Black Combe area which, together with south-west Cumberland, Borrowdale (Keswick) and Cross Fell, has recently been re-examined.

In next Monday's *Nature Physical Science*, Helm and Roberts describe in detail the latest information about the Skiddaw Slate-Borrowdale Volcanic junction. But there is still much doubt about the detailed succession and the faulting and folding in the Skiddaw Slates.

clarify the issue of whether energy amplification is necessary at some stage in chemosensory transduction. Many of the speakers implied that it is, and this is clearly a crucial problem.

The low threshold for the response of many insect antennae to sex attractants is now well known. Dr R. A. Steinbrecht (Max Planck Institute für Verhaltensphysiologie, Seewiesen) explained how the silk moth antenna collects molecules of sex attractant, bombykol, and how these molecules reach the receptor cell membrane, and Drs K. E. Kaissling and G. Kasang (also of Seewiesen) discussed its remarkable isomeric specificity and its ability to resolve brief pulses of sex attractant applied in series. The properties of this receptor mechanism are rivalled in specificity by those of the antenna of the moth *Plusia ni* in response to its sex pheromone, cis-7-dodecenyl acetate. Dr L. K. Gaston (University of California, Riverside) spoke of the enormous differences in responses by this preparation when stimulated by different isomers of the compound, and similar ones of differing hydrocarbon chain length. Other speakers, including Dr J. Jakinovich (University of Michigan, Ann Arbor) on fly chemoreceptor specificity in response to different isomers of inositol, and Dr W. A. Kafka (Seewiesen) on the relative responses of locust olfactory cells to different isomers of methylbutanoic acid, revealed astonishingly narrow specificities. But few clues have emerged about the structure of the receptor sites for any of these pheromone molecules.

Studies were also presented on aspects of response characterization of sensory cells of vertebrate nasal and lingual epithelia, on the innervation of these cells, and on the way in which their responses may be influenced through intravascular stimuli. Perfused cyclamates can generate responses which summate with those to sweet tasting substances applied externally to the rat tongue (Dr R. M. Bradley, Nuffield Institute for Medical Research, Oxford). Another interesting study presented by Dr B. Oakley (University of Michigan, Ann Arbor) indicates that sets of taste papillae of the rat tongue, which show similar response characteristics, become associated with branches of the same afferent nerve fibre. This may imply that nerve fibres growing out into the tongue "search for" and recognize papillae of similar specificity pattern.

The proceedings of the symposium are due to appear in a volume of collected papers in about six months time, and it was agreed that the next symposium in this series would be held in Melbourne, Australia, about three years hence.

THE EARTH

Chondritic Again

from our Geomagnetism Correspondent

THE idea that the principal minor element in the Earth's fluid outer core may be sulphur rather than either nickel or silicon—and thus that the Earth as a whole may be chondritic after all—has now received further substantial support from Hall and Murthy (*Earth Planet. Sci. Lett.*, **11**, 239; 1971). This thesis, which Murthy and Hall propounded last year (*Phys. Earth Planet. Interiors*, **2**, 276; 1970) on the basis of an analysis of the relative abundance of volatile elements in the crust and mantle, was more recently taken up by Lewis (*Earth Planet. Sci. Lett.*, **11**, 130; 1971) who adduced several pieces of indirect evidence for it and discussed in some detail its important consequences. But Lewis's admitted difficulty was the lack of the thermodynamic data needed to make the case really convincing. It is this deficiency which Hall and Murthy have now remedied to a limited extent.

The classical view of the formation of the Earth's core is that an iron-nickel mixture segregated from a silicate matrix. It is Hall and Murthy's contention, however, that the first melt to form during the early history of the Earth was an FeS-Fe eutectic. These

different processes are clearly likely to have different effects on the distribution of elements throughout the Earth and thus imply different courses for the Earth's subsequent history. So how would the elements be distributed if an Fe-S liquid had separated to form the Earth's core? In an attempt to answer this question Hall and Murthy have taken seventeen "diagnostic" elements, including the alkali metals Li, Na, K, Rb, and Cs, the alkali earths Be, Mg, Ca, Sr, and Ba, the transition metals Mn and Fe, and other critical elements such as Cu, Zn, U, Th, and Pb, and have tried to determine thermodynamically how they would become distributed between a predominantly Mg-Fe silicate matrix and an Fe-S melt during the Earth's early history.

As a starting point Hall and Murthy present thermodynamic data for the simple reaction $\text{MeO} + \text{FeS} = \text{MeS} + \text{FeO}$ where Me is an electropositive element. It is quite clear from the table they have compiled that K, Rb and Cs have high negative free energies of reaction at both 298 K and 1,000 K and thus preferentially form sulphides. These data thus support Lewis's contention that if an Fe-S liquid segregates into the core it will take with it a great deal of the available K, Rb and Cs, so that these elements will accordingly be depleted in the crust relative to a chon-

The Micro-friction of Graphite

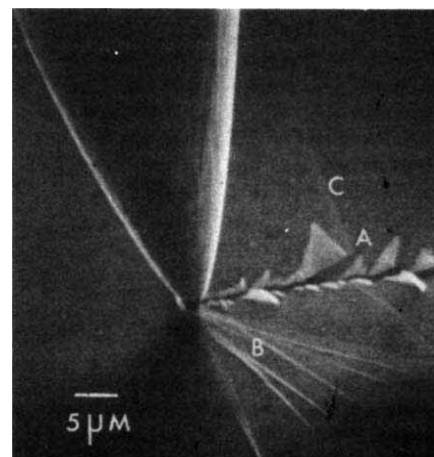
NEW frictional studies of graphite at low loads (1 to 400 mg) have revealed much greater differences between the coefficient of friction of the basal and edge planes than expected. In next Monday's *Nature Physical Science*, Skinner, Gane and Tabor report their measurements of the coefficient of friction, using a scanning electron microscope and a delicately loaded tungsten stylus with a tip radius of about 1 μm .

They have found that the coefficient of friction on the edge plane is between fifteen and sixty times greater than the coefficient measured on the basal plane (0.3 compared with about 0.005 to 0.02); this is a much larger anisotropy than has been measured previously. Skinner *et al.* suggest that this is because their experiment involved not only small loads but also almost perfect surfaces which were carefully selected under the electron microscope.

At loads less than 40 mg, the low coefficients of friction of the basal plane were usually accompanied by only slight surface damage and, in some cases, by no damage at all. Sometimes the cleaving of a flake was observed as in the figure and very occasionally a

peeling action took place; this was associated with much higher coefficients of friction (about 0.4).

At greater loads the basal planes were often too weak to support the stylus and even when they did, the stylus was found to penetrate further and further into the specimen until the experiment had to be stopped.



Scanning electron micrograph of a stylus sliding on the basal plane of graphite. A, Flaking of the surface; B, peeling; C, a cleavage step.

ditric composition. On the other hand, it would be unrealistic to suppose that such a simple reaction is applicable to the whole Earth and thus that the oxide-sulphide equilibrium is anything but vaguely indicative of real conditions. This view is supported by the data. For example, sodium can also be seen to possess a high negative free energy of reaction, which fact would suggest that sodium was also withdrawn into the core and correspondingly depleted in the crust. But this is patently not so.

As Goldschmidt pointed out many years ago (in *Geochemistry*, Clarendon Press, Oxford, 1954), different reactions are applicable to different parts of the Earth. Thus Hall and Murthy go on to consider more appropriate equilibria. For the crust they examine reactions of the type MeSO_4 with FeS and MeCO_3 with FeS , which are consistent with the typically high oxidation states of crustal material. By contrast, for the mantle, which at the time of Fe-S segregation would have been in a reducing environment, the corresponding reactants with FeS are of the type MeSiO_3 and Me_2SiO_4 . For each of the seventeen elements and for each reaction Hall and Murthy have computed heats of reaction (which dominate the free energies of reaction).

The results are striking. Elements like Be and Th (and thus by implication U) are strongly lithophilic under both crustal and mantle conditions. By contrast, elements such as Cu, Hg, Ag, Pb and Zn are strongly chalcophilic under crustal conditions and remain so throughout the mantle. But the critical elements K, Rb and Cs which are strongly lithophilic under crustal conditions rapidly change to being chalcophile under mantle conditions. In the presence of an Fe-S liquid these elements would thus have been drawn into the liquid and thus into the core. Sodium, on the other hand, remains lithophilic in the primitive mantle—and so the anomaly implied by the oxide-sulphide equilibrium disappears. In short, notwithstanding the fact that the reactions considered must be oversimplified compared with those applicable to the real Earth, the thermodynamic data obtained by Hall and Murthy are consistent with known abundances of the relevant alkalis in the crust and mantle.

As Hall and Murthy admit, however, this apparent success conceals some real objections. For example, their calculations have been made for an aluminium-free system; and it is well known that Na and K have different terrestrial relationships with Al. Under crustal conditions both Na and K form aluminosilicates, especially feldspars. But in the mantle, where pressures are higher, feldspars are unstable and change

to denser phases such as jadeite, the Na aluminosilicate. Potassium, on the other hand, does not apparently produce a high pressure form equivalent to jadeite for Na. If such a form really cannot exist, that fact alone may account to some extent for the different behaviours of Na and K in mantle regions. Ignorance of mantle conditions at the time of core formation precludes any detailed assessment of the role of Al at present, although, whatever the cause, the difference in behaviour between Na and K seems established. And further support for it comes from crystal chemistry. Potassium (and Cs and Rb) ions are larger than Na (and Li) ions and are thus less likely to fit into the mantle pyroxene structure but more likely to be absorbed into an available liquid. Thus Hall and Murthy suggest that "the incompatible nature of K, Hb, and Cs which has been reflected in their selective enrichment into basaltic melts through geological time must also be reflected in their enrichment into an Fe-S liquid during core formation in the early history of the Earth".

The distribution of elements implied

by the early existence of an Fe-S melt, and, in particular, the removal of K into the core, would, of course, have affected the Earth's thermal history—and this implies that many conventional thermal models of the Earth must be revised. The apparent near-constancy of K/U ratios for a variety of terrestrial rocks has led many people to suppose that such a ratio (about 1×10^4) is a fixed feature of the Earth, the critical variable being the original total radioactivities in various parts of the Earth. But what Hall and Murthy have done is to show that this assumed "coherence" between K and U is not necessarily valid and that, on the contrary, it is quite possible that K separated from U (and Th) during the early history of the Earth.

Finally, the fact that Rb behaves like K in the presence of an Fe-S liquid would explain why Rb/Sr ratios in the Earth's crust are lower than chondritic. In short, if Hall and Murthy are right about the presence of an Fe-S melt during the early history of the Earth, the principal objections to the chondritic Earth are apparently removed.

Dynamic Stereochemical Model for ATP Synthesis

THERE have been many models of the mechanism by which chloroplasts and mitochondria can utilize redox energy associated with electron flow for photo and oxidative phosphorylation. In recent years the chemiosmotic scheme and chemical hypothesis (see Greville in *Current Topics in Bioenergetics* (edited by D. R. Sanadi, 3, Academic Press, New York and London, 1969) have commanded the most attention. These schemes, however, tend not to deal with the detailed molecular processes which must be involved.

In next Wednesday's *Nature New Biology*, A. Bennun presents an hypothesis which attempts to relate molecular conformational changes within proteins with the process by which electron flow gives rise to ATP synthesis. He considers that the proteins involved in the energy transduction are coupling factors isolated from either chloroplasts (CF_1) or mitochondria (F_1) which show ATPase activity under appropriate conditions. These factors are thought to undergo dynamic stereochemical reactions induced by electron transport which involve charge dependent conformational changes.

Bennun has used kinetic data from chloroplast studies (Bennun and Avron, *Biochim. Biophys. Acta*, 79, 646; 1964) to produce a scheme which involves two stages. The first stage is the formation of a proton dependent electrostatic complex, which is produced, it is argued, when an electron carrier, closely associated with CF_1 or F_1 , is reduced.

Because of the change in electric field the redistribution of the electrons within the coupling factors produces new dipolar forms. This complex, however, is not thought to be capable of directly inducing phosphorylation until the second stage, which is brought about by a loss of a proton and the dissipation of the partial charges within the protein. Bennun argues that the disappearance of the dipolar structure of CF_1 or F_1 leads to the formation of a phosphorylated high energy intermediate which then gives rise to ATP. The formation of these complexes is suggested to occur within the membrane which, as Bennun points out, would tend to result in structural restriction of the conformational changes associated with charge redistribution. Such restrictions could then act as effective energy sources.

Bennun takes his model one step further in attempting to explain the mechanism by which membrane located ATPase catalyses the hydrolysis of ATP and utilizes the energy for active transport of ions. Essentially the reverse process to that already described is thought to occur. The protons released could conceivably conserve their energy by binding to the relevant proteins which may be the ATPase itself or some other membrane protein. This binding could induce conformational changes involving stretching dipoles similar to that suggested for the coupling factors, and may occur as a result of changes in the degree of hydration of the protein.

Supermassive Objects in Astrophysics

VIRGINIA TRIMBLE

Institute of Theoretical Astronomy, Cambridge.

The scientific interests of William A. Fowler cover an unusually wide range. The symposium held at the Institute of Theoretical Astronomy, Cambridge, on July 19 to 21, 1971, in honour of his sixtieth birthday was designed to explore some of the topics with which he has been concerned and the relationships among them.

It was the extraordinarily large amounts of energy needed to explain radio sources and quasars that first led Hoyle and Fowler^{1,2} and Hoyle, Fowler, Burbidge and Burbidge³ to suggest that single objects much more massive than stars might be important in astrophysics. Since then, supermassive stars have been in and out of fashion several times as sources of energy and sites of nucleosynthesis, each time motivating a variety of theoretical studies and suggesting new ways of looking at observational data. Speakers at the symposium repeatedly emphasized the importance of the interplay of observations, theory and laboratory experiments in shaping our understanding of massive objects as well as ordinary stars and the processes occurring in them.

Black and White Holes

In accordance with the Fowler dictum of always searching for the objects with the largest possible binding energy, a session was devoted to collapsed configurations. The work of Penrose^{4,5}, Israel⁶, Carter⁷ and Hawking (to be published) has led to a picture of gravitational collapse in which the formation of a closed, trapped surface (roughly, one from which no light ray or other signal can ever escape to infinity) is followed inevitably by the formation of a singularity in the curvature of space-time. This singularity is probably shielded from view by a horizon, forming a "black hole", although the existence of what Penrose calls naked singularities cannot be excluded. Their work has also shown that space-time outside the singularity probably comes to look (asymptotically as time goes on) like either the Schwarzschild solution or the Kerr solution to Einstein's field equations. These solutions are characterized respectively by the mass of the singularity and by its mass and angular momentum. The singularity may also be charged but all other properties (lepton number, baryon number, multipole moments of the electromagnetic field and so forth) are probably lost or radiated away during the collapse. Thus, in the words of J. A. Wheeler, a black hole has no hair.

B. Carter (University of Cambridge) reviewed recently proven theorems directed towards changing the probabilities in the above picture to certainties. Not all loopholes have yet been closed.

S. Hawking (University of Cambridge) has shown⁸ that there is a limit to the fraction of the mass energy of a singularity that can be radiated in all forms from a collision with another singularity. This limit is always less than 50%. Thus black hole collisions cannot be a 100% efficient source of gravitational radiation in a particular bandwidth, even if each object is involved in a series of collisions. This increases the amount of mass that must be involved in producing the pulses of gravitational radiation reported by Weber⁹.

Despite the horizon surrounding a Kerr-type singularity, it is possible to extract that part of its energy associated with rotation rather than rest mass. J. A. Wheeler (Princeton University) suggested this as one of the most promising ways of finding observational evidence for black holes. (Searches for gravitational lens effects produced by them, X-rays resulting from matter accreting on them, gravitational radiation emitted at their formation, and binary systems with black hole components have also been proposed, and in some cases attempted.) Wheeler considered the fate of a white dwarf or neutron star falling into the neighbourhood of a black hole of mass $\sim 10^5 M_{\odot}$. Tidal forces may fracture the star into two or a few pieces in that region known as the ergosphere (where the dragging of inertial frames by the rotating singularity is so rapid that no particle can remain at rest as seen by an observer at infinity). Then (as discussed by Penrose¹⁰ for a single incident particle) while one fragment falls into the singularity, another can come out with more total energy than the incident star originally had. About 30% of the total mass-energy of a black hole with the maximum possible angular momentum for its mass could be extracted in this fashion.

W. D. Arnett (Rice University and Cambridge) provided a certain amount of discouragement by reporting a series of calculations of the late phases of evolution of a star of mass 30 to 100 $M_{\odot}$. In no case was a dense remnant left which was significantly larger than the Chandrasekhar limit for stable white dwarf stars. Thus only if the upper limit to neutron star masses is smaller than that for white dwarf masses would any of these remnants ever collapse to a black hole.

White holes are the time reversals of black holes, that is, mass energy expanding out of a singularity. They can thus provide literally unlimited amounts of energy. The apparently complete arbitrariness of the time at which the expansion starts was partially responsible for the lack of enthusiasm for white holes expressed by many of the symposium participants.

Supermassive Stars and Disks

The relativists participating in the symposium clearly regarded collapse to a singularity as a desirable event. The astrophysicists, on the other hand, were principally concerned with preventing supermassive stars from collapsing, or at any rate from collapsing too quickly, so that their energy could be extracted in some useful form. The problem is that there are, once one has taken relativistic effects into account, no stable, cold, slowly rotating configurations for masses greater

than one or two $M_{\odot}$. And even the "hottest" configuration of a spherical supermassive star burning nuclear fuel is not stable against collapse above about $10^6 M_{\odot}$ —whether the star is assumed to be static or undergoing relaxation oscillations. Possible additional sources of support to balance gravitation in still larger objects are magnetic fields and rotation. The latter is particularly useful: there is no limit to the amount of mass that can be put into an infinitely thin, rotating disk. The problem in this case is that the disk seems to fragment into a number of small objects, and this process and subsequent events are very difficult to calculate. F. Hoyle (University of Cambridge) and E. Salpeter (Cornell University) each reviewed a probable "life history" of a supermassive object from formation to fragmentation and/or ultimate collapse.

Very massive stars may have formed out of the general interstellar medium early in the history of the galaxy, but this does not seem to be occurring at the present time. The most likely (or at any rate least unlikely) point of origin for a supermassive star now is at the centre of a galaxy or star cluster. Here, the gas lost by stars (either peacefully or through supernova explosions) will collect and condense, provided it can dissipate enough of its angular momentum. If the gas falls in sufficiently quickly, creating turbulence, dissipation of its dynamical energy will heat the gas and raise its entropy. If opacities are high enough to trap all the energy, pressure will suffice to support a spherical equilibrium configuration (which may or may not be stable) of mass up to $10^8 M_{\odot}$. Slower collapse will lead to a rotating disk because the entropy of a given mass of interstellar medium is not as great as that of a spherical supermassive star of the same mass.

The supermassive star continues to lose energy by radiation and to contract. Its centre heats up and, for masses up to $10^6 M_{\odot}$ (if there is no rotation) or $10^8 M_{\odot}$ (for the maximum amount of uniform rotation), nuclear reactions "turn on" before the star reaches the critical radius at which its binding energy ceases to increase making collapse inevitable.

I. Appenzeller and K. Fricke (University of Göttingen), R. Talbot (Rice University and Cambridge) and R. Tayler (University of Sussex) presented results of calculations of nuclear burning in stars of mass 100 to $10^7 M_{\odot}$. The German group finds that the relaxation oscillations proposed by Fowler¹¹ will probably occur and prevent collapse in stars of masses $\gtrsim 0.75 \times 10^6 M_{\odot}$. The star first contracts: its central temperature and density, and thus the rate of nuclear energy generation, increase rapidly until the star is lifted back out again and the energy generated is released as thermal and non-thermal radiation and kinetic energy of matter blown off. The star then contracts again and so forth. The oscillation period is about one year, which might be relevant to periodicities perhaps observed for quasars. Objects more massive than $10^6 M_{\odot}$ will not have their collapse halted by this process.

For stars with masses of 10^2 to $10^3 M_{\odot}$, R. Talbot and Papeioizou (University of Sussex) find that pulsational instability almost certainly does not blow the star apart because pulsation energy is dissipated in shock waves and fed into stable harmonics of the unstable fundamental mode. Rather, material seems to be lost gradually. As this process continues, layers are lost which were previously in the star's convective core. They thus contain material in which nuclear reactions have occurred. Stars of this type early in the history of the galaxy, could have contributed an appreciable fraction of the helium as well as heavier elements we now observe. (Stars less massive than $50 M_{\odot}$ or so appear to contribute very little helium.)

These objects can yield, at best, 0.01 of their rest mass energy in useful form, because that is the limit of efficiency of nuclear processes. To do better, we must consider more condensed configurations, whose binding energy is a significant fraction of Mc^2 . Because of the collapse that sets in, for a massive spherical object below $r \sim 3 \times 10^5 \left(\frac{M}{M_{\odot}}\right)^{3/2}$ cm (where the binding energy is still $\ll Mc^2$), this means considering a

rotating disk or a dense swarm of smaller objects.

The binding energy of a uniformly rotating, thin disk—most of the results thus far obtained will carry over to differentially rotating disks—increases monotonically with its degree of compactness to a maximum of about $0.37 Mc^2$ at the point where the redshift of light from its centre is infinite^{12,13}. Such a disk is therefore probably stable against overall gravitational collapse and thus useful for astrophysical purposes.

J. Bardeen (University of Washington) discussed the optical appearance of such a disk. As it contracts, it develops an ergosphere from which energy can be extracted. The effect of the rapid rotation on photons emitted is roughly to blue-shift those emitted in the forward direction of the rotation and redshift those emitted in the backward direction. This effect is superimposed on the gravitational redshift produced by any massive object. Thus, although some photons are received at infinity with a blueshift, the total luminosity of the object goes to zero as the redshift from its centre goes to infinity. Light from the disk has a unique redshift only if it is uniformly rotating and seen face on. Most of the photons which reach a distant observer are, however, emitted in the equatorial plane of the disk, making such a disk by itself unpromising as a quasar model.

A. G. W. Cameron (Yeshiva University) discussed the probable behaviour of a gas disk inside a dense star cluster. The winding up of magnetic field lines by differential rotation may lead to the ejection of jets of material along its rotation axis by a mechanism analogous to that found by Leblanc and Wilson¹⁴ for supernovae. Second, even a small amount of gas in this form can turn cosmic ray energy into thermal radiation very efficiently. Finally, accretion of the gas onto stars, by raising their masses, may appreciably enhance the supernova rate in the cluster.

Contributions by F. Pacini (Frascati) and by L. M. Ozernoi and V. V. Usov (Lebedev Institute, Moscow) dealt with massive rotating objects with magnetic fields. These might be expected to exhibit behaviour somewhat like that of pulsars, which somehow convert rotational energy to a useful form very efficiently. Some rough analogies between the properties of quasars and those of the Crab Nebula add interest to this type of model.

The contraction of a mass of $10^9 M_{\odot}$ from an initial radius of 10^{21} cm, initial rotation period of 3×10^8 yr, and initial magnetic field strength of 10^{-5} to 10^{-6} gauss (reasonable properties for the centre of a galaxy) to a radius of 10^{16} cm yields a rotation frequency $\Omega \sim 10^{-7} \text{ s}^{-1}$ and a magnetic field strength $B \sim 10^4$ to 10^5 gauss if angular momentum and magnetic flux are conserved. The rotation energy of such an object is $\sim 10^{60}$ erg and its magnetic energy $\sim 10^{56}$ erg. The magnetized disk then radiates by non-thermal as well as thermal mechanisms^{15,16}, contracts, and speeds up its rotation.

If the dominant form of energy loss is by magnetic dipole radiation at the rotation frequency, or particle ejection, then the luminosity of the object increases as $(1 - t/t_0)^{-4/3}$ and its rotation frequency increases as $(1 - t/t_0)^{-1/2}$. Such low frequency electromagnetic radiation cannot, of course, propagate in any reasonable surrounding medium, but there is, in any case, of order

$$L = 10^{47} \left(\frac{B}{10^5}\right)^2 \left(\frac{R}{10^{16}}\right)^6 \left(\frac{\Omega}{10^{-7}}\right)^4 \text{ erg s}^{-1}$$

of energy made available in some form. L may be decreased by a factor $\sin^2 \theta$ if magnetic and rotation axes are not perpendicular.

The time dependence of the luminosity is more complicated if there are other important energy losses or if the magnetic axis gradually becomes aligned with the rotation axis but the observed number of quasars, active galactic nuclei and the like as a function of luminosity is not inconsistent with the prediction of even a simple model. The luminosity of the disk stops

increasing and drops rapidly to zero as seen by an observer at infinity when the disk has shrunk to such an extent that relativistic effects become important.

R. Wagoner (Cornell University and Cambridge) discussed the fate of the electromagnetic moments of a disk having a distribution of azimuthal currents and charges (such that the net charge of the disk is zero) on it, when the disk collapses as a result of the extraction of angular momentum from it. The magnetic dipole and octapole and electric quadrupole moments all appear to vanish as $(Z_c + 1)^{-2}$ as Z_c , the redshift of light coming from the centre of the disk, goes to infinity. There is no reason to believe that other multipoles will not do the same. Thus, only visible objects seem able to radiate by the pulsar mechanism.

A black hole continues to exert a gravitational force on the outside world and thus can have a rotating disk around it, made up of material which either did not collapse or was accreted after the collapse. Lynden-Bell has considered such disks as the energy sources for quasars and galactic nuclei^{17,18}. Viscosity in the disk will cause the gas to spiral gradually into the black hole. By the time a particle reaches the last stable orbit outside the black hole, it will have lost 42% of its rest mass energy at infinity. This is the most efficient way of extracting energy from ordinary matter so far found.

The thin, uniformly rotating disk considered by Salpeter, Bardeen and Wagoner^{12,13,19} seem to be stable against collapse (though not, of course, against contraction as they radiate energy away). They are not, however, stable against fragmentation. Although gravitational forces are balanced by rotation and thermal forces (gas and radiation pressure) for the disk as a whole, gravitation predominates strongly for a piece of the disk having a size much greater than the thickness of the disk but much less than its radius, when this piece is considered by itself. As the disk loses energy, it becomes flatter, and, when it reaches an axial ratio of 0.05 (for a uniformly rotating disk), it will probably break into two or a few pieces. These, in turn, radiate away energy and flatten until they are unstable to breakup, and so forth.

Although the swarm of fragments continues to occupy about the same volume as the original disk did, they are individually much denser than it was. Thus binding energy has been extracted from them. Each generation of fragments is able to radiate the same fraction of its total energy. In many generations, the process can therefore become quite efficient.

The smaller objects will differ from the original disk in having higher equilibrium central temperatures, so that nuclear burning will turn on at some point, delaying the contraction and fragmentation of the blobs. They will also be increasingly efficient emitters of magnetic dipole radiation (provided some magnetic field was initially present) if total angular momentum and magnetic flux are conserved as they fragment. Gravitational radiation will also become increasingly important as fragmentation progresses if the mass distribution maintains its asymmetry.

There are many complications in this process, for example, variations in the angular momentum distribution, possible collisions of blobs, possible re-coalescence of blobs, dissipation of angular momentum by viscosity, dissipation of asymmetries in the mass distribution by gravitational radiation, and possible ways of extracting the thermal energy of the gas. It is just possible that the fragmentation could end in a swarm of (essentially) mass points whose gravitational potential is sufficiently flat at the centre to produce a fairly constant gravitational redshift from a central gas cloud, thus permitting objects with large redshift to be closer to us than their cosmological distances, as suggested by Fowler and Hoyle²⁰.

F. Hoyle (Cambridge) pointed out that, despite the wide range of phenomena possible in this scheme in which objects proceed toward more and more condensed states, it may not suffice. We may yet have to consider matter evolving through expansion especially to explain violent events in galactic nuclei and the formation of large double radio sources.

Observations

The need for large amounts of energy ($\sim 10^{62}$ erg) to account for the observed properties of radio sources first suggested to Hoyle and Fowler that supermassive objects might be important in astrophysical contexts. Observations since that time have, with about equal frequency, suggested new theoretical approaches to the problems of quasars and galactic nuclei and created difficulties for old ones.

G. Burbidge (University of California, La Jolla) and R. Weymann (Arizona) reviewed some of these problems, ranging from very general questions of energetics to specific difficulties, such as whether or not emission line profiles of Seyfert galaxies can be understood in the context of a gas disk rotating around a condensed central mass.

A compilation of the various kinds of radiation and mass loss which we see going on²¹ suggests that the release of gravitational energy in the nuclei of galaxies may be the most important source of energy in the universe. Some more or less continuous process must be involved, since nearly all nuclei show a degree of activity. This continuous activity might be easier to understand as the result matter expanding from, rather than contracting to, a condensed state or singularity.

It also appears difficult to explain the ejection of coherent, massive objects from galactic nuclei on the basis of the conventional contraction hypothesis (although either Wheeler's ergosphere mechanism or ejection of bits of Salpeter's fragmented disk could impart large velocities to bodies of mass $\lesssim 1 M_\odot$). Among possible evidence for such massive coherent ejecta are tiny radio source components far from their parent galaxies (including those in Cygnus A, ref. 22), the bright optical blobs in the jet of M87, and the Seyfert-like galaxies VV 144 and I Zw 95 which are connected by jets or bridges to nearby, smaller, more normal looking objects. On a less spectacular scale, asymmetries and transient doubling of Seyfert galaxy emission lines can be attributed to the ejection of blobs of gas of mass 0.1 to $1.0 M_\odot$ with velocities of 1,000 to 5,000 km s⁻¹.

Actual or potential observations can also have a bearing on whether a single, supermassive object or a swarm of smaller ones is more likely to be responsible for active nuclei. Genuine periodicity in the variations of the brightness of quasars and so on would be strong evidence for a single, coherent rotating object (although differential rotation is likely to shear off a "hot spot", so that a given pattern of variability would only last for a few periods²³). The large amplitudes of quasar light variations already suggest that one or a few objects are responsible for most of the continuum light output at a given time. A series of radio outbursts along the same axis or a single outburst involving more than $\sim 10^{52}$ erg (the binding energy of a neutron star) would also be evidence for a single object. A single, supermassive magnetic rotator might be detectable as a low frequency, very small radio source in the way that the Crab Nebula pulsar was first seen²⁴. Finally, an outburst from a single central object might be expected to disturb and blow away the thermal gas cloud which produces the emission lines in an active nucleus. The Seyfert galaxy NGC 3516, in which a broad H β line disappeared sometime between 1942 and 1967 (ref. 25) and reappeared between 1967 and 1969 may have done something of the sort.

In 3C 120, on the other hand, the broad hydrogen lines appear not to have been disturbed by the sharp radio outbursts which have occurred in the past few years. This would seem to be evidence for a group of smaller objects, only one of which blew up (or perhaps for a single object with strong directivity). If successive violent events in a number of small objects are responsible for the luminosity of a small, bright Seyfert nucleus like that of NGC 4151, then the centre of light ought to move around. This also applies to the radio positions of unresolved quasars.

The amount of energy and mass required to explain violent

activity in quasars and galactic nuclei depends, of course, on their distances. Distances to peculiar objects are measured either by direct application of the Hubble–Lundmark relation or from their physical association with other objects whose distances we think we know. The problems arise when these conflict. For ordinary galaxies, selected by some appropriate luminosity criterion, the redshift–magnitude relation is fitted extremely well by a straight line. For quasi-stellar objects, on the other hand, it is a scatter diagram²⁶; thus we lack strong evidence that redshift is a measure of distance for these objects.

Several cases have been found in which two objects with different redshifts appear to be connected by bridges, jets or arms. These include NGC 4319+MK 205, NGC 7603+ a nearby compact galaxy, and IC 1746+ an adjacent quasi-stellar object. In other cases (for example, VV 172 and Seyfert's Sextet) objects with different redshifts are not connected but are clustered at least in the plane of the sky. Finally, Burbidge, Burbidge, Solomon and Strittmatter (to be published) have found that four of the 3C quasars are within 6' of spiral galaxies in de Vaucouleur's catalogue. Only 0.2 such close association would be expected by chance. On the other hand, one or more QSOs appear to be associated with normal galaxies having the same redshift²⁷. Some of the participants were sufficiently impressed to express the view that conventional physics may be inadequate to explain these data.

The optical continuum of an active nucleus should reveal something about the objects producing it; if we see the surface of a supermassive star or disk directly, then the continuum spectrum ought to be basically thermal whereas a non-thermal spectrum would imply that the energy of the central object is being transformed into relativistic particle and magnetic field energy.

As observed, the optical continuum of active objects typically looks non-thermal. It may, however, be necessary to correct the continuum for the reddening found by Wampler²⁸ for Seyfert emission lines. This will be the case if the material producing the lines covers all or most of the continuum source. After making such a correction, Pagel (Royal Greenwich Observatory) and Kaneko²⁹ find that the emitted spectrum in fact looks like the Rayleigh–Jeans part of a thermal continuum, with $T \gtrsim 60,000$ K, rising into the ultraviolet.

As for line spectra, Seyfert and Markarian type nuclei are easier to understand than QSOs. The galactic nuclei typically show relatively sharp forbidden emission lines and hydrogen Balmer lines with sharp cores and much broader ($\Delta V \sim 5,000$ km s⁻¹) wings. Along a sequence from Seyfert galaxies to QSOs, the ratios [OIII]/H β and H β (core)/H β (wings) tend to drop (to zero in some cases).

Photoionization appears able to account for all the lines observed in Seyfert nuclei, as the presence of [FeXIV] in NGC 4151 has not been confirmed³⁰. The forbidden lines are necessarily produced in a low density region, while the broad Balmer lines are most easily understood in terms of a combination of electron scattering in dense, small cores ($N_e \sim 10^8$ to 10^9 ; $R \sim 10^{16}$ cm) and ejection of line-radiating blobs at velocities of 1,000 to 5,000 km s⁻¹. A model with rotational broadening due to an optically thin disk rotating around a central mass $\gtrsim 3 \times 10^8 M_\odot$ is also possible, but only a very narrow range of parameters can account for the observed profiles. No abundance anomalies are required to explain Seyfert galaxy line spectra, but abundance variations by factors of 2 or 3 are quite possible.

In quasars, on the other hand, no model appears to account satisfactorily for the line profiles. If resonance lines are trapped efficiently enough to yield the observed widths, then they should be strongly self-reversed. And sufficient gas motions to smear out the self-reversal would also make the forbidden lines broad.

As far as abundances are concerned, it is still not clear whether the weakness of helium recombination lines relative

to those of hydrogen in 3C 273 is an abundance effect. It might, rather, be due to a low degree of helium ionization³¹. A better upper limit to, or detection of, HeI (5876 Å) would resolve the question.

Objects like NGC 1068, whose Balmer lines do not have broad wings, may have had their dense gas swept out by violent events at their centres. If the events take the form of ejection of relativistic particles, Rayleigh–Taylor instability at the particle–gas interface would produce filamentary structure like that seen in NGC 1275. The gas would also be given the sorts of velocities found in transient line doubling and would eventually produce larger regions of more tenuous gas. These are seen (with diameters ~ 100 pc) in Seyferts and perhaps in QSOs if the relatively sharp QSO absorption lines are produced in the objects themselves.

We may be observing one object in transition between the broad, supernova-like absorption bands seen in PHL 5200 (ref. 32) and the sharp absorption lines, often at several very different redshifts, seen in other QSOs. The object PHL 957 (ref. 33) has an emission redshift of 2.679 (as measured on Lick spectrograms; E. M. Burbidge, private communication) and at least eight separate absorption redshifts. One of these is represented by extremely broad Ly α and Ly β lines at $Z = 2.308$. This is the largest difference so far found between an emission and an absorption redshift for broad lines, and neither the large ΔZ nor the broadness of the lines can be understood in terms of intergalactic absorption. The object is thus evidence for a high velocity explosion in a high density region of the QSO.

Nucleosynthesis and Reaction Rates

The rates of nuclear reactions are crucial to the determination of what nuclear fuels are burned, at what temperatures and densities they burn, and what the reaction products are, both in supermassive and ordinary stars. C. A. Barnes (California Institute of Technology) pointed out that the completeness of our knowledge declines rapidly with increasing atomic weight of the reactants. All the cross sections needed to deal with hydrogen burning seem to be well established but at the other end of the scale, it is not even obvious what nuclear information is required in order to treat silicon burning. The abundances of the products of silicon burning may, in some cases, depend on nuclear reaction rates and, in others, on equilibrium considerations and binding energies.

In the intermediate range, after the conversion of hydrogen to helium, the next nuclear reactions in stars are (a) the combination of three alpha particles to form carbon-12, (b) the addition of another alpha particle to form oxygen-16, and (c) successive heavy ion reactions to form neon-20, magnesium-24 and so on. Since it appears that the rates of the reactions of type (c) are much slower than (a) and (b), the relative abundances of carbon and oxygen depend almost exclusively on the rates of (a) and (b). Recent information^{34–36} (and unpublished work by Barnes, Dyer, Kavanagh, Mann, McCaslin, Morgan, Nichols, Thompson and Weissner at California Institute of Technology) leads to the conclusion that the time is rapidly approaching when a comparison of the observed carbon to oxygen ratio with that predicted from laboratory measurements will provide a critical test of prevailing theories of helium-burning nucleosynthesis. At the same time, the carbon–oxygen ratio at the conclusion of helium burning appears to be of great importance for later, explosive stages of stellar evolution, possibly even determining whether or not a remnant is left after such an explosion or supernova.

A knowledge of the nuclear reactions that occur in stars of various masses and the birth rates of these stars as a function of time in the Galaxy could be combined to give the history of the nuclear composition of the Galaxy provided we knew how much of each star is thrown back into the interstellar medium for reuse and how much is trapped in white dwarfs, neutron stars, and black holes. Calculating this history there-

fore requires three input parameters, all of which are extremely uncertain; nor are the data on stellar compositions as a function of age and position in the galaxy, with which the calculations are to be compared, as well established as we would like. Nevertheless, such calculations have been attempted by W. D. Arnett (Rice University and Cambridge) and A. G. W. Cameron (Yeshiva University) and their colleagues. The two groups have made rather different assumptions, especially about the fractions of a star going into the various possible reaction products (unprocessed hydrogen + proton enriched nuclei, helium, carbon and oxygen, iron, neutron enriched nuclei, white dwarfs, neutron stars and black holes). Each group has succeeded in matching the observations of metal abundance as a function of stellar age. Cameron has also taken account of the constraints on the production of heavy nuclei provided by solar system data on the abundances of radioactive nuclei and their decay products. Arnett's work also reproduces the distribution of stellar abundances as a function of position in the galaxy.

Insight is provided by these calculations into some details of observed stellar abundances. For instance, the systematic tendency of the ratio N/Fe to be very low whenever Fe/H is low (B. Pagel), follows from the fact that nitrogen is largely produced in CNO cycle burning of hydrogen, which requires that carbon and oxygen have been produced in a previous generation of stars. Thus no nitrogen is formed until the second generation.

It is necessary in these nucleosynthesis calculations to assume that a larger fraction of star forming material went into massive stars in the past than it does now. Otherwise, according to Cameron, almost 25% of the one solar mass stars now seen would have metal abundances less than 10% of the solar value, which is not the case. Thus galaxies should have been much bluer and much brighter in the past.

There is, however, some observational evidence that the birthrate function, $\Phi(M)$, does not change its shape as a function of time. W. L. W. Sargent (California Institute of Technology), L. Searle, and W. Bagnuolo have found that the present *UBV* colours of Irregular and Sc galaxies can be understood in terms of a birthrate function given by $\Phi(M, t) = A(t)M^{-n}$. If a typical galaxy is 10^{10} yr old, then $n=1.35$ for Sc and 1.1 for Irregular type galaxies. For the bluest galaxies of each type, $A(t)$ has been essentially constant, whereas for the reddest ones (for example, Magellanic Cloud type Irregulars), $A(t)$ is now about 0.1 of its value 10^{10} yr ago.

Even at the present time and for our own Galaxy, $\Phi(M)$ is not well known, especially for very high and very low masses. The power law M^{-n} , must cut off (for $n > 1$) below some minimum mass; otherwise the integral mass involved would be infinite. Where this cutoff occurs is important because low mass stars lock up both processed and unprocessed material and prevent it from taking further part in galactic nucleosynthesis. Because objects with $M \lesssim 0.1 M_{\odot}$ never burn hydrogen, they are almost undetectable except as astrometric companions to nearby dwarf stars (for example, Barnard's star). This means that the low mass cutoff may be unobservable. The very massive stars, which contribute the largest fraction of heavy elements, are also a problem. N. J. Woolf (Minnesota) noted that the brightest (and therefore presumably most massive) single star known (6 Cyg No. 12, $M_{\text{bol}} \lesssim -10$) is hidden behind a dense envelope, seriously hampering our determination of the space density of such stars. A large, wide field telescope in the southern hemisphere should make it possible to determine the birthrate function for stars more massive than 1 to $2 M_{\odot}$ in the Magellanic Clouds where the effects of dust obscuration are much less severe than in the Galaxy.

Finally, it is not even clear that our Galaxy is a closed system as assumed in all calculations of galactic nuclear evolution. It is quite possible that the clouds of neutral hydrogen considered by Oort^{38,39} to be falling into the galactic plane at high velocities represent intergalactic matter

being added to the interstellar medium at a rate of 0.1 to $1 M_{\odot} \text{ yr}^{-1}$. Such matter would presumably consist of only hydrogen and perhaps helium and would thus significantly dilute the heavier elements produced in stars.

Concluding Remarks

A number of problems remain, particularly in understanding how supermassive objects are formed. Nor is there yet any really firm observational evidence for their existence. But when supermassive stars were first proposed as a source of large amounts of energy, they seemed to be pulsationally unstable. Subsequent work, some of it discussed at this symposium, has shown that this is probably not the case. Thus we are perhaps justified in joining the Fathers of the Subject, Hoyle and Fowler⁴⁰, in turning a blind eye, a deaf ear, and a cold shoulder to written, oral and implied criticism respectively.

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South Labrador Sea and the Evolution of the North Atlantic

A. S. LAUGHTON

National Institute of Oceanography, Wormley, Godalming, Surrey

Basement morphology and magnetic anomalies in the South Labrador Sea have preserved the history of the changes from a two-plate configuration of sea floor spreading to a three-plate configuration after the split between Greenland and Rockall Plateau and back to a two-plate configuration when the Labrador Sea stopped widening.

BEFORE the separation of Rockall Plateau and North-West Europe from Greenland and the growth of the Reykjanes Ridge, the Labrador Sea was the northern part of the Atlantic Ocean. Although it is floored by sediments^{1,2} which almost totally obscure the basement topography except south of Greenland, a buried mid-ocean ridge has been located beneath the sediments by seismic and magnetic methods²⁻⁶. The identification of the magnetic anomalies on the Heirtzler time scale⁷, and hence the history of the sea during the Mesozoic and Tertiary has, however, been uncertain^{2,6,8}.

This article reports a new interpretation of the South Labrador Sea based on data obtained during leg 12 of the Deep Sea Drilling Project in D.V. Glomar Challenger and earlier data.

Basement Morphology

Fig. 1 shows what is now known of the basement in the southern Labrador Sea, and includes some features typical of seafloor spreading, such as mid-ocean ridges, median valleys and transform faults. At 57° N, 48° W, Le Pichon *et al.*² have identified a buried median valley which is consistent with the magnetic data in the area. Further east, at 57° N, 43° W, two lines of basement ridges are separated by an east-west valley filled with sediment, which appears to be a median valley paralleled by magnetic anomalies⁹. On this meridian, this is the only place between the Gibbs Fracture Zone and Cape Farewell where the basement topography suggests a spreading centre (see Fig. 11 of ref. 10). The adjacent ridges both seem to have been uplifted and tilted away from the axis preserving a sediment cover of 0.2 s on their outer flanks. These two sections of median valley are not colinear and are separated by a broad ridge complex about 70 km wide striking north-east-south-west. The ridge can best be interpreted as a fracture zone or transform fault offsetting the axis of the median valley. The strike of the transform fault is in general agreement with the pole of rotation of 61° N, 92° W calculated for the last phase of the opening of the Labrador Sea by Le Pichon *et al.*².

South of Greenland, Le Pichon *et al.* found basement ridges which they interpreted as a transform fault (the Farewell Transform Fault) associated with the first phase of opening of the Labrador Sea. Further south, they also associated the buried part of the Gibbs Fracture between 45° and 47° W with this phase, and from these two features the pole for the first phase of opening was computed to be at 80° N, 89° W.

The remaining structural trend is normal to the west end of

the Gibbs Fracture Zone, and is perhaps truncated by it, suggesting that the ridges on this trend are the remains of structure parallel to the spreading axis during the first phase of opening of the Labrador Sea. Magnetic analyses indicate that the ridges may be the extinct spreading axis. Further north-west, the ridge at 55.7° N, 48.5° W, is parallel to the median valley to the north of it and probably belongs to the second phase of opening.

All the basement trends in Fig. 1, except the exposed part of the Gibbs Fracture Zone, can be correlated with either the spreading axes or transform faults associated with the two phases of opening of the Labrador Sea. The Gibbs Fracture Zone¹¹ east of 43° W is associated with spreading about the Mid-Atlantic Ridge further east.

The semicircle formed by the basement ridges has been a barrier to deep water circulation and the transport of sediment. It encloses a large body of sediment typified by topographic

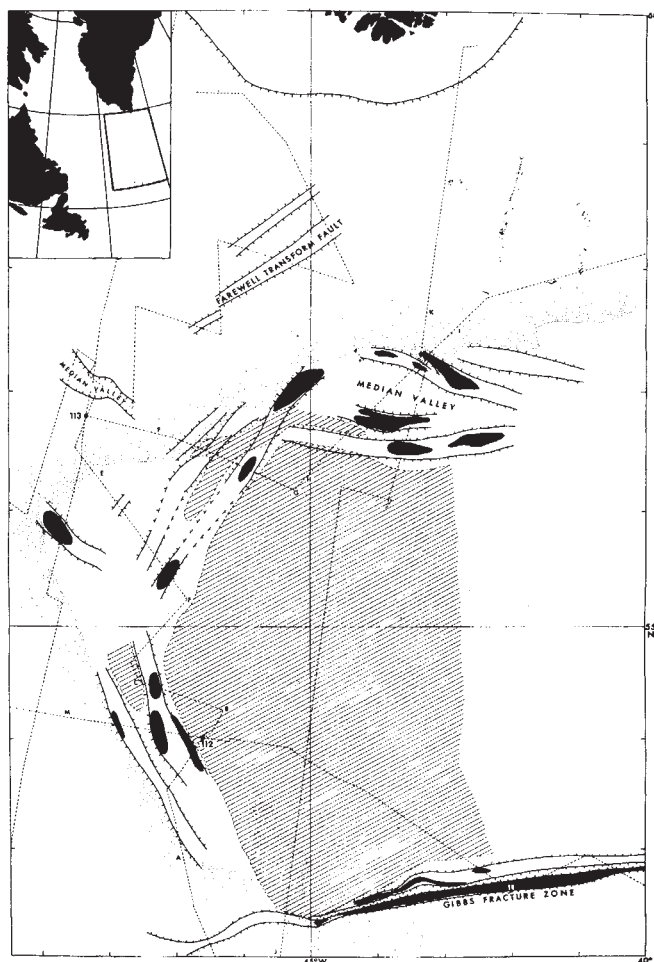


Fig. 1 Basement structure and sediment cover of the South Labrador Sea derived from seismic reflexion profiles and from bathymetry. Ticked lines=basement scarps; dense hachure=basement exposures on sea floor; stippled boundary=contact between terrigenous sediments and bottom current controlled sediment body; light hachure=area of oligocene mid-sediment reflector.

ridges several hundred kilometres long, a wavy surface, marginal channels and moats, and no conformity with the basement. It is relatively transparent except for a mid-sediment reflector (shaded in Fig. 1) shown by drilling to be Oligocene. These characteristics suggest that the sediments were laid down under the influence of the near bottom currents^{8,10,12} and the pattern of ridges indicates an anticlockwise bottom circulation fed from the east through the Gibbs Fracture Zone. From the extent of the reflector and the conformity with it of overlying beds, the deep circulation in the southern Labrador Sea seems to have remained relatively constant since the mid-Tertiary in spite of the considerable change in width of the Atlantic.

Some of the sediments have overflowed the basement barriers and to the west and south are lying underneath the terrigenous sediments which have accumulated at a high rate due to the proximity of land and which were sampled at site 113; their extent is shown in Fig. 1 by the stipple boundary. Another sedimentary supply by ocean currents along the south-east coast of Greenland gave rise to the Eirik Ridge¹² which lies above the Farewell Transform Fault. Contrary to the suggestion of Jones *et al.*¹⁰, this does not seem to be related to the sediment body at 55° N, 45° W.

Magnetic Data

There is a symmetrical pattern of magnetic anomalies, which trends north-west-south-east in the Central Labrador Sea and east-west south of Cape Farewell^{1,2,4-6,9,13,14}, offset by two fracture zones trending north-east-south-west. The axis of symmetry in the central Labrador Sea is magnetically smooth, but two groups of high amplitude anomalies (500 gamma peak-to-peak) lie halfway between the axis and the continental slope. South of Cape Farewell, the southerly group of these

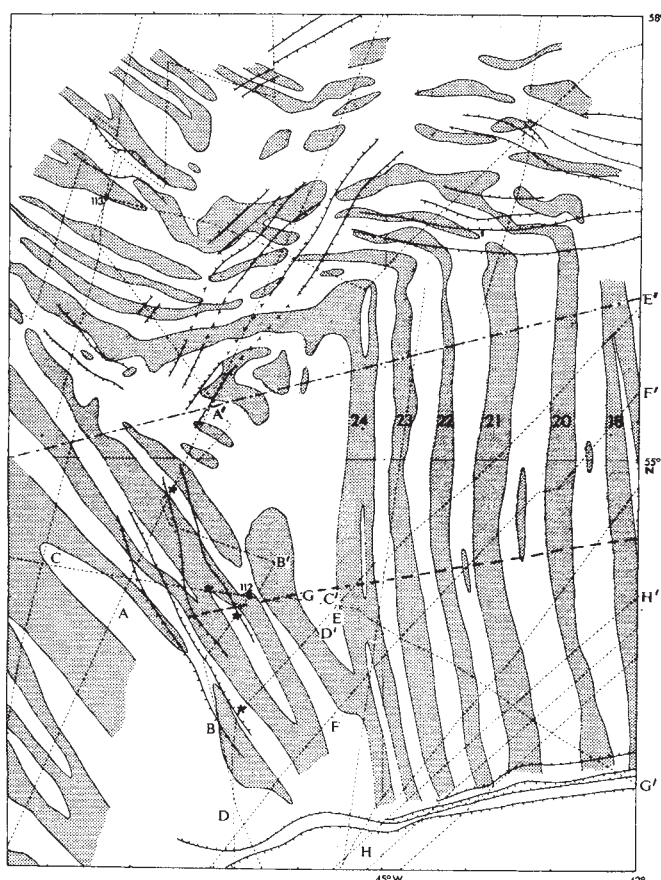


Fig. 2 Magnetic anomaly chart of South Labrador Sea. North of dot-dash line, contours are modified from Vogt *et al.*¹⁵. South of this line, control is by tracks shown. Shaded area is positive. Anomaly numbers are from Fig. 3.

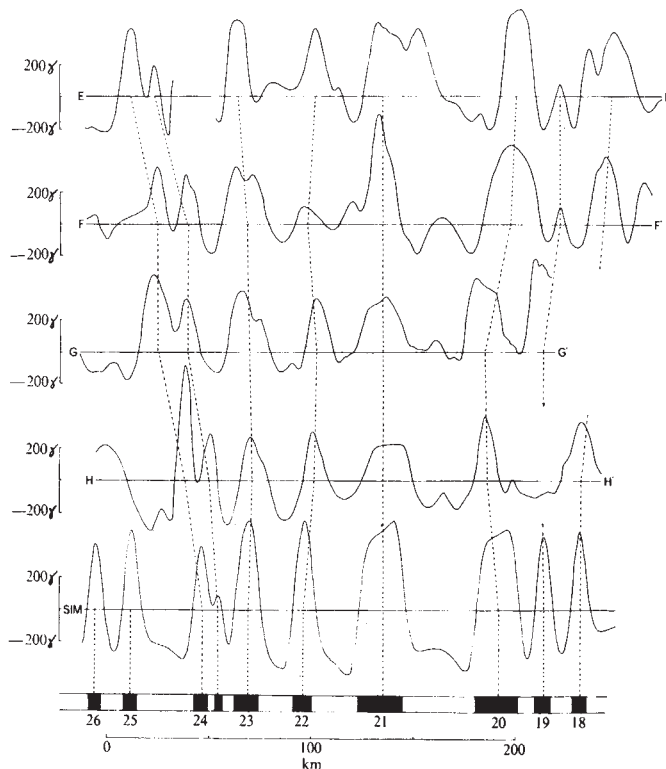


Fig. 3 Magnetic anomalies along tracks EE', FF', GG' and HH' (Fig. 2) projected perpendicular to anomaly trend 170°, compared with simulated anomaly using Heirtzler *et al.*⁷ time scale for anomalies 18 to 26. (Magnetized layer 4.5 to 6.5 km below sea level; effective susceptibility ± 0.01 c.g.s. units; black blocks normally magnetized. Spreading rate of 1.26 cm yr^{-1} .)

anomalies bends sharply southwards parallel to the Mid-Atlantic ridge. At 52.5° N they are offset eastwards by the Gibbs Fracture Zone. The interpretation of the tectonic history of the Labrador Sea depends critically on the age of these anomalies and of the axis.

In the South Labrador Sea, the magnetic anomalies (Fig. 2) relate closely to basement trends and reflect the various phases of opening of the Labrador Sea and the North Atlantic. North of 56° N, the contours (modified from Vogt *et al.*¹⁵) show the trends parallel to the median valleys, and the symmetry about them, and the offset due to the transform fault. The strong north-south lineations between 42° and 45° W can be matched with a simulated anomaly pattern for anomalies 18 to 24 (ref. 7) with a spreading rate of 1.3 cm yr^{-1} parallel to the Gibbs Fracture Zone (Fig. 3). Some dislocation of this pattern may, however, be present along the heavy dashed line in Fig. 2, which is a westward continuation of the fracture zone crossing the Mid-Atlantic Ridge at 53.5° N (ref. 16).

West of 45° W, the magnetic anomaly pattern trends north-west-south-east, parallel to the basement ridges near site 112. The correlations in this area are less certain than the north-south ones, but the trend is clear and continues to the north-west into the area of better mapped anomalies in the Labrador Sea. Near site 112, the lack of continuity may be due to the fracture zone. The same north-west-south-east trend is repeated towards the south-west as far as the continental margin. Further evidence of a change of pattern west of anomaly 24 comes from the absence of anomalies 25 and 26 on the projected profiles in Fig. 3. A triangular area centred on 55° N, 46° W, for which there are no data, is enclosed by the three prominent trends.

Data Interpretation

We can now follow the evolution of the northern part of the North Atlantic with some certainty by working backwards using the above magnetic data together with other magnetic

data from several sources (refs. 14, 17–20 and data from the USNOO) and the fracture zones (Fig. 4). The principal fracture zone controlling the North Atlantic opening is the Gibbs Fracture Zone, known also as the Charlie or 53° N Fracture Zone (refs. 11, 16, 21 and data from the NIO and the USNOO). South of this are numerous fracture zones offsetting the ridge axis to 28° W and indicated by earthquake epicentres. These have been lumped together as one fracture zone at 50° N.

The symmetry of magnetic anomalies 19 to 24 about the Mid-Atlantic Ridge between 53° and 56° N confirms the identification of the north-south anomalies south of Greenland. The continuity of anomaly 20 between a north-south orientation and an east-west orientation at 56.5° N, 43° W (Fig. 2), its proximity to the median valley, and the apparent absence of such a right angle for anomaly 19, suggests that the Greenland and Labrador plates have not separated appreciably since anomaly 19 (47 m.y. ago). On this assumption, the European plate can be closed into the Labrador-Greenland plate using the Gibbs Fracture Zone as a control guide to give the 47 m.y. (Middle Eocene) palaeogeography (Fig. 5). A good fit of anomaly 19 is obtained. A consequence of the immobility of Labrador-Greenland plate boundary after 47 m.y. is that the median valley south of Cape Farewell should not extend further east than 41.5° W; this can be tested by further surveys.

Before 47 m.y., Greenland and Labrador have to be closed along the lines indicated by the fracture zones offsetting the Mid-Labrador Sea Ridge axis, while the closure of the Reykjanes Ridge still continues. The anomaly 24 or 60 m.y. (Palaeocene) palaeogeography (Fig. 6) differs slightly from that presented by Vogt *et al.*⁹ in the greater counterclockwise rotation of the European plate. Rockall Plateau, shown by holes 116 and 117 to have subsided from above sea level since the Palaeocene, is not close up against the continental slope of Greenland at this time although anomaly 24 is very close to Rockall Plateau. The original Greenland continental margin may, perhaps, have been downwarped after the break-away of Rockall Plateau or else an Upper Cretaceous geo-

synclinal sedimentary basin may have existed before the split. In the central Labrador Sea, the two prominent magnetic anomaly groups are apparently older than 60 m.y.

At 60 m.y., the whole anomaly pattern changed from the two plate geometry because of the separation of the European plate from Greenland. All magnetic anomalies older than 60 m.y. have a trend more north-west-south-east than those of 60 m.y. and younger. These trends were directed parallel to the Labrador continental margin and approximately parallel to that of the Greenland-European plate margin. The most prominent of these trends are those near site 112 which are now median to the Palaeocene ocean and offset from the central Labrador Sea anomalies by the Farewell Transform Fault and its counterpart on the Labrador margin². This transform fault may be part of the line of weakness continuing north-east into the region between Rockall Plateau and Greenland which subsequently split to give the Reykjanes Ridge. The anomalies near site 112 and the associated basement ridges may be part of the fossil ridge axis resulting from the final phases of the pre-Palaeocene spreading which took place before a shift of stress pattern occurred beneath the plates. The initiation of spreading between Rockall Plateau and Greenland profoundly modified the geometry by the introduction of a triple junction and switched the spreading axis near site 112 from north-west-south-east to north-south.

If this theory is correct, the age of the seafloor near site 112 should increase towards the north-east, up to the angle of anomaly 24, and the magnetic anomaly pattern across the ridge should be symmetrical. Some profiles, projected at right angles to the ridge, are presented in Fig. 8. Some crude symmetry can be seen about an axis between two positive peaks. Only profile AA' is long enough without course alterations to test the symmetry in detail beyond the positive peaks. The symmetry axes are indicated in Fig. 2 by asterisks, and do not lie in a straight line along the ridge. Either the detailed contouring of the ridge (both by basement topography and by anomaly) is wrong or there are undetected fracture zones offsetting the old axis.

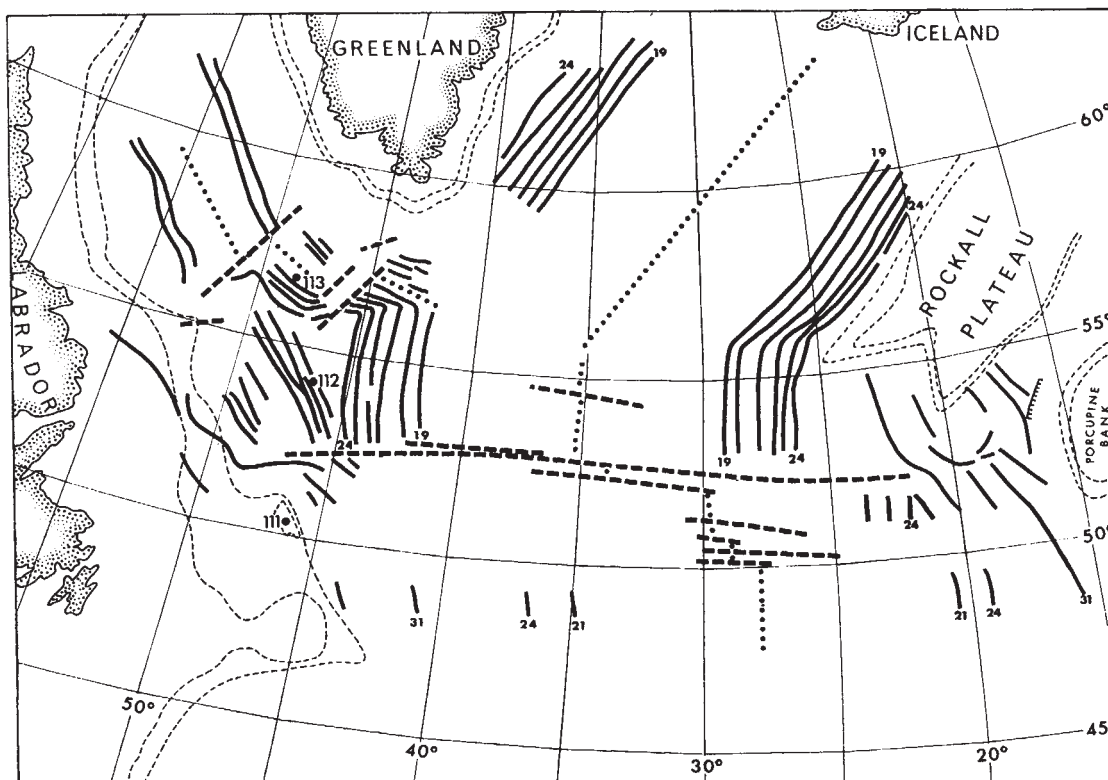
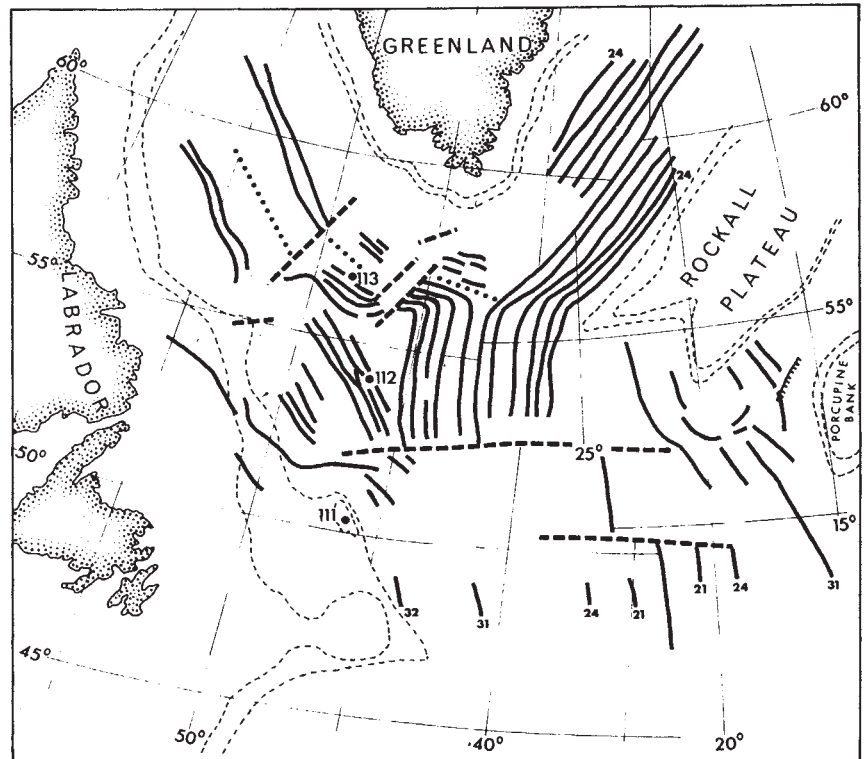


Fig. 4 Magnetic anomaly trends in the North Atlantic. ---, Top and bottom of continental slope; —, positive magnetic anomalies from number 19 and older; ---, fracture zones; ..., present and extinct spreading axes.

Fig. 5 Palaeogeographic reconstruction at anomaly 19 (47 m.y., Middle Eocene).



The age of the basement sampled at site 112 is calculated to be about 65 m.y. from the ages of the sediments sampled; that is, about anomaly 26. The magnetic profiles are, however, not long enough to compare a synthetic model with the observed profiles and thus to test this age against the Heirtzler time scale. The absence of large anomalies to the north-east of 112 agrees with the suggestion that the positive peak near the site is anomaly 26, in which case the central negative anomaly would be at 64 m.y. The switch of spreading axis therefore probably took place during the period 64 to 60 m.y. ago.

If the western end of the Gibbs Fracture Zone is a transform fault², the pre-Palaeocene spreading centre to the south of it must be further east. The eastward end of this transform fault might be found in the south-eastern margin of Rockall Plateau and the anomalies perpendicular to this may be parallel to this spreading centre. A second, parallel fracture zone may be linked to the south end of the Jean Charcot fault²² in Rockall Trough. The suggestion that the south end of Rockall Trough has resulted from north-east-south-west transform faults bounding north-west-south-east magnetic anomalies is contrary to previous ideas that a spreading axis lay parallel to the trough.

Further evidence for the switch in orientation of the spreading axis comes from the magnetic anomaly pattern immediately south of the east end of the Gibbs Fracture Zone at 51° N, 22° W (Fig. 4). Anomaly 24 runs north-south along 22° W whereas, immediately to the east of this, the anomaly trend is north-west-south-east. This north-west-south-east anomaly is associated with a pronounced basement ridge similar in many ways to that at site 112, which may therefore be a remnant of the pre-Palaeocene spreading centre to the south of the Rockall Trough transform fault. Similar remnants have been reported to the south of the Gibbs Fracture Zone in the western Atlantic by Le Pichon (personal communication).

Reconstruction

A tentative reconstruction (Fig. 7) can be made for anomaly 31 (72 m.y. or Late Cretaceous) using (a) the three suggested transform faults, and (b) the position of spreading centres of (i) the central Labrador Sea in the same position as after

60 m.y., (ii) the southern Labrador Sea on the ridge near site 112 and (iii) the Atlantic on a median line between anomalies 31 on either side. The continuity of anomaly 31 south of Rockall Plateau²⁰ suggests that the transform faults between 50° and 53° N did not exist at this time and that the spreading axis was therefore inclined to the 60 m.y. year spreading axis.

It is difficult to take this reconstruction further back in time. But clearly Greenland can be made to fit into Labrador in the position indicated by Bullard *et al.*²³. With Rockall Plateau attached to Greenland, no problems of overlap would

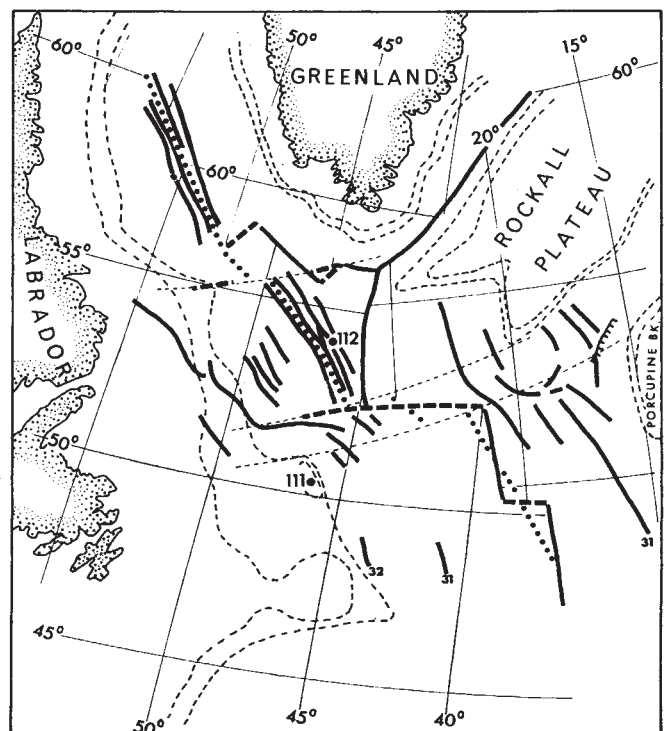


Fig. 6 Palaeogeographic reconstruction at anomaly 24 (60 m.y., Palaeocene). — — —, Possible small circles of transform faults; . . . , spreading axes before 60 m.y. ago.

ensue, but the east-west and north-south scarps at the south-west end of Rockall Plateau would not be explained. The wide separation of anomalies between the two ends of the two transform faults associated with Rockall Trough suggests that they are older than anomaly 32 (76 m.y.) and would imply that the true continental margin off Labrador at 51° N is towards the shelf break. It does not explain the continuation of Rockall Trough to the north-east. South of this, both Porcupine Bank in the east, and Orphan Knoll and Flemish Cap in the west appear to be partially detached pieces of continent which need to be restored to their original position before this region can be reconstructed. Anomaly 32 (76 m.y.) places a minimum age on the separation of Europe from Canada. Linear extrapolation of the spreading rate between anomaly 31 and 32 back to the Labrador shelf edge at 49° N indicates that its age is 82 m.y.

The stratigraphic history of Orphan Knoll (site 111) shows that during the Late Cretaceous (100 to 70 m.y. ago) the sediments deposited there were neritic. By the Maestrichtian (70 m.y.), Orphan Knoll had subsided to about 200 m depth, whereas before 100 m.y. it was probably non-marine. This is consistent with an age of splitting of Europe from Labrador of about 80 m.y. Appreciable subsidence of Orphan Knoll to oceanic depths did not, however, take place until the early or middle Tertiary, by which time a considerable width of North Atlantic Ocean had been formed. The adjustment of the continental margins to an equilibrium state after the initiation of an oceanic split seems to take several tens of millions of years, a time constant that may reflect the creep of lower crust or upper mantle in response to non-isostatic conditions. Alternatively the late subsidence may be the result of later tectonic activity.

This reconstruction follows closely that suggested by Le Pichon *et al.*², but differs in suggesting that Rockall Trough did not open as a spreading axis, but as part of an earlier transform fault. A slight revision of Le Pichon's timing gives the sequence of events: (1) 82 m.y.: European plate (with Rockall Plateau and Greenland) breaks from Labrador pulling off fragments of Porcupine Bank, Flemish Cap and Orphan Knoll; (2) 82 m.y. to 60 m.y. (anomaly 24): Labrador Sea

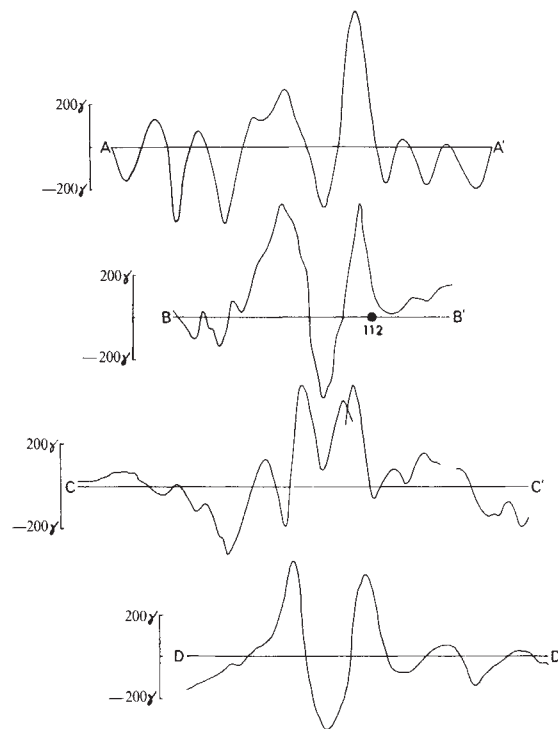


Fig. 8 Magnetic anomaly profiles AA', BB', CC' and DD' (Fig. 2) across ridge near site 112, projected perpendicular to trend 330°.

and North Atlantic open; (3) 60 m.y. (anomaly 24): Rockall Plateau breaks from Greenland initiating a triple junction and a shift of spreading axes and fracture zones to accommodate the new geometry; (4) 60 m.y. to 47 m.y. (anomalies 24 to 19): simultaneous opening of Labrador Sea, Reykjanes Ridge and North Atlantic; (5) 47 m.y. (anomaly 19): Greenland stops moving relative to Labrador, and Labrador Sea growth finishes; (6) 47 m.y. to present (anomalies 19 to 0): Reykjanes Ridge and North Atlantic grow as European plate separates from Greenland-Labrador plate¹⁵.

These ideas arose out of discussions with the scientific party of leg 12 of the Deep Sea Drilling Project, whom I thank. I also thank many institutions and scientists who contributed unpublished data and manuscripts to leg 12. Especially I would like to acknowledge the magnetic data prepared by R. H. Higgs and P. R. Vogt of the US Naval Oceanographic Office. P. R. Vogt, W. C. Pitman and X. Le Pichon provided helpful criticisms.

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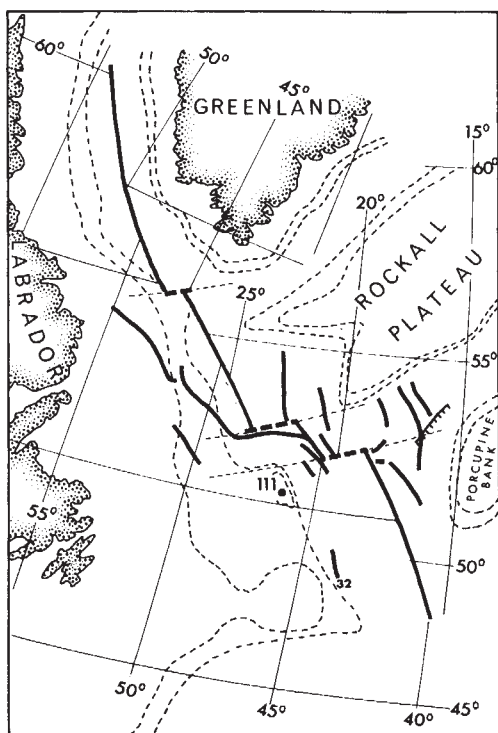


Fig. 7 Palaeogeographic reconstruction at anomaly 31 (72 m.y., Late Cretaceous). [] [] [], Jean Charcot fault²².

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C-Type RNA Tumour Virus Genome Expression in Wild House Mice

MURRAY B. GARDNER, J. EARLE OFFICER & ROBERT W. RONGEY

Department of Pathology, University of Southern California School of Medicine, Los Angeles, California

JOHN D. ESTES, H. C. TURNER & ROBERT J. HUEBNER

Viral Carcinogenesis Branch, National Cancer Institute, Bethesda, Maryland

The presence of the indigenous latent C-type RNA tumour virus genome can be demonstrated in wild house mice by detection of gs antigen and C-type particles. There seems to be strong suppression of the activity of this viral genome in this feral species.

ALTHOUGH laboratory strains of mice have been subjected to extensive cancer virus investigations, wild house mice (*Mus musculus*) have received limited attention^{1–4}. There are no reports of this isolation of murine leukaemia and sarcoma viruses nor of C-type virus particles from wild house mice, but recently our associates have reported the presence of group specific (gs) antigens of the murine C-type viruses in the tissues of wild mice bred in captivity⁵. Since the genome of this species has been demonstrated in all laboratory strains of mice tested^{5–7}, we set out to look for the murine C-type viral genome in feral wild house mice.

In 1968, we began a study in Los Angeles County (a) to detect and determine the natural history of the C-type tumour virus expression in wild mice and (b) to study the effect of chemical carcinogens on the expression of the viral genome. Here we report our findings on the prevalence of detectable gs antigen by complement fixation and of C-type particles by electron microscopy in tissues from recently trapped wild mice and from mice kept in captivity for 16–26 months, some of which have recently developed spontaneous tumours, and from young and old mice following tumour induction by 3-methylcholanthrene (MC).

Treatment of Mice

About 600 wild mice were trapped alive in urban Los Angeles, weighed and individually housed in mason jars. Several hundred mice were killed without treatment and 180 were set aside without treatment to live out their life span (geriatric

colony). The remainder were equally divided by sex into three treatment groups. One group of 150 mice was inoculated subcutaneously with 300 µg and another group of 50 mice with 150 µg of MC dissolved in 0.05 ml. of trioctanoin. A control group of 146 mice received a 0.05 ml. subcutaneous dose of trioctanoin alone. Sera, collected from all mice by retro-orbital puncture immediately after capture and at the time of tumour collection, were tested for antibodies in complement fixation against eleven murine viral antigens*. Ten per cent sonicated aqueous extracts of (a) spleen from the untreated mice, (b) spleen from the control trioctanoin injected mice and (c) tumour and spleen from mice with induced or spontaneous tumours were tested for complement fixing murine gs antigen by the microtitre technique⁸ using, as antiserum, a 1:20 dilution of serum pools from Fisher rats bearing transplanted sarcomas induced by the Moloney strain of murine sarcoma virus (MSV)⁹.

Sections of tumour and spleen were also prepared for light and electron microscopy. At the time of collection of each MC-induced tumour a trioctanoin injected control mouse was killed and the tissues similarly processed. The time of initial appearance of MC tumours was noted but tumours were allowed to reach 25–35 mm in size before the animal was killed unless they showed evidence of ulceration or necrosis. The average weight of the mice at the time of trapping was 14 g, ranging from 8–19 g.

Methylcholanthrene Indices

No significant differences were observed in tumour incidence or latent period before tumour appearance between the 150 µg and 300 µg MC treatment groups, and so these two groups are combined in Table 1. After intervals of 12–15 months, ninety-eight of 135 (73%) surviving mice which had been inoculated with MC had developed tumours at the inoculation site. The latent period varied from 75–458 days with an average of 143 days. Most of the tumours were fibrosarcomas; several had

* Lymphocytic choriomeningitis, murine adenovirus, polyoma, murine hepatitis virus, reovirus-3, K virus, Theiler's GD7 virus, Kilham rat virus, syncytial virus-5, Moloney sarcoma virus (MSV) and Sendai virus.

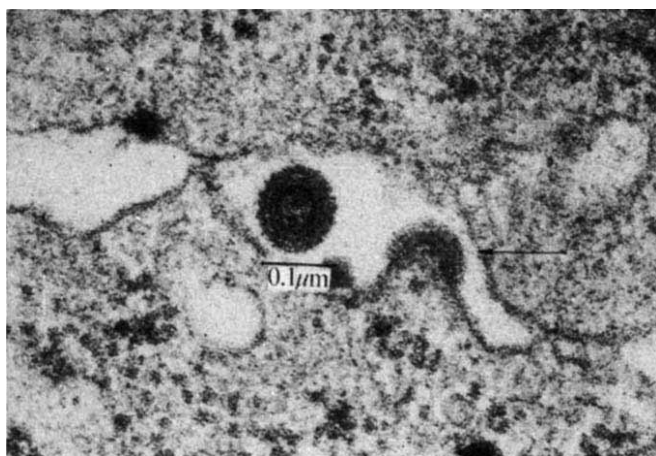


Fig. 1 One budding (arrow) and one extracellular 100-nm C-type virus particle in spleen from a wild house mouse bearing an MC-induced sarcoma. The sarcoma from this mouse also contained C-type particles. (Uranyl acetate; $\times 105,750$.)

features of rhabdomyosarcomas and eleven were squamous cell carcinomas. In two mice both a carcinoma and a sarcoma occurred at the inoculation site. Tumours were not observed by gross or microscopic examination in 146 surviving trioctanoin injected control mice observed up to 18 months.

All inoculated mice remained free of antibodies to the eleven indigenous murine viruses. When we used a highly specific but narrow range MSV rat serum pool (No. 12), selected to contain antibodies reactive only with the species specific major (gs-1) antigen¹⁰, we found antigen in low titre (1 : 2–1 : 4) in six of fifty-eight (10%) tumours tested and in none of the corresponding spleens; but spleen extracts from seven of fifty (14%) recently trapped untreated wild mice also contained gs antigen. In the trioctanoin inoculated control group, two of sixty-one spleen extracts contained gs antigen. Many of these same tissue extracts were thawed after storage for 1 yr and retested with a more broadly reactive MSV rat serum pool (No. 21, see below); positive CF reactions were observed in fourteen of forty-four (32%) MC tumours, ten of forty-four (23%) of the corresponding spleens, none out of twenty-five of the spleens from trioctanoin control mice and thirteen of forty-two (31%) of spleens from untreated control mice (not shown in Table 1).

C-type particles (Fig. 1) were found by electron microscopy in both the MC tumour and corresponding spleen from eight of sixty-six (12%) mice examined. Seven of these tumours were fibrosarcomas, one a squamous cell carcinoma, and all contained detectable gs antigen using the narrow range serum pool (No. 12). C-type particles were not found in any of twenty spleens from the trioctanoin control group. A few were

observed in three of twenty-one spleens randomly chosen from the freshly trapped untreated group (see below). Intracisternal A-type particles (Fig. 2) were found in nineteen MC induced tumours, six of which were squamous cell carcinomas and thirteen of which were fibrosarcomas; no C-type particles were detected in these tumours.

In a second experiment (Table 2), twenty old wild mice from the geriatric colony, which had been kept in captivity without treatment for 15 months, were inoculated subcutaneously with 200 μ g of MC. Nine of sixteen surviving mice developed sarcomas at the inoculation site after an average latent period of 100 days, when the mice were estimated to be 19–20 months of age. One mouse without a tumour at the inoculation site had a granulosa cell tumour of the ovary.

At this time an MSV rat serum pool of intermediate sensitivity (No. 18) was used for complement fixation tests; this serum pool contained antibodies against the species specific major component (gs-1) and, at lower titres, antibodies against an interspecies cross reacting component (gs-3)¹¹ of the gs antigen complex. Five of nine (55%) tumour extracts from these old mice contained gs antigen compared with four of thirty-one (13%) MC-induced tumour extracts from young adult mice tested with the same rat serum pool No. 18 (Table 1); several of the tumours from older mice were positive at titres higher (1 : 4–1 : 16) than those observed in younger mice with tumours induced by MC. In the older mice C-type particles were present in two of eight MC-induced sarcomas and in spleens of the same animals. Complement fixation antigen was detected in two of seven spleen extracts and C-type particles were seen in two of eight spleens from normal old mice which had been kept in captivity without treatment for 20 months. All of the old mice also remained free of antibodies to the eleven indigenous viruses described above.

Spontaneous Tumours in Old Wild Mice

Tumours have occurred in sixteen of 150 surviving old mice that had been under observation without treatment for 19–26 months (Table 3). Eight of these tumours were lymphomas, four were fibrosarcomas and four were large pulmonary adenomas. The lymphomas were lymphoblastic and characterized primarily by splenomegaly and generalized lymphadenopathy without thymic involvement. Two of the lymphomas arose in mice subjected 1–2 yr previously to unsuccessful transplants of MC-induced wild mouse sarcomas; the remaining tumours were unquestionably spontaneous. Using more broadly reactive MSV serum pools (Nos. 21 and 25) which contained high titres of antibodies to species specific, interspecies gs antigen^{12,13} and possibly additional antigens, complement fixing reactions were detected in high titre (1 : 4 or above) in the spleen and/or tumour in all eight lymphomatous mice, and in lower titre (1 : 2–1 : 4) in two of the fibrosarcomas and in each of the pulmonary adenomas. C-type particles were observed in five

Table 1 Tumour Incidence, Murine C-Type Tumour Virus gs Antigen and C-Type Particle Expression in Young Adult Wild Mice Untreated and after Inoculation with Methylcholanthrene

Treatment	Tumours †	Tissue	CF gs antigen *		C-type particles	A-type particles
			MSV-12	MSV-18		
Methylcholanthrene, 150 or 300 μ g subcutaneously	98/135 ‡	Tumour	6/58 §	4/31	8/66	19/66 ¶
		Spleen	0/60	2/31	9/61	5/61
Control—trioctanoin	0/146	Spleen	2/61		0/20	2/20
No treatment—killed immediately	0/121	Spleen	7/50		3/21	1/21

* The MSV rat serum pools utilized differed in sensitivity but not specificity for the murine C-type tumour virus gs antigen. MSV-12 was least sensitive and MSV-18 was of intermediate sensitivity (see text). The mice tested with MSV-18 were different from those tested with MSV-12.

† Number of tumours/number of surviving mice.

‡ Eighty-seven sarcomas and eleven squamous cell carcinomas (including two cases of combined carcinoma and sarcoma); period of observation 12–18 months. Latent period range 75–458 days with an average tumour latent period of 143 days.

§ Number positive/number tested free of anticomplementary activity.

|| All eight tumours and six of the corresponding spleens were positive for complement fixing antigen; seven of these tumours were fibrosarcoma and one a squamous cell carcinoma.

¶ Six of the nineteen tumours with A-type particles were squamous cell carcinomas; three of the five spleens with A-type particles were from mice with squamous cell carcinomas.

Table 2 Tumour Incidence, Murine C-Type Virus gs Antigen and C-Type Particle Expression in Old Wild Mice Untreated and following Inoculation with Methylcholanthrene

Treatment	Tumours†	Tissue	CF gs antigen * MSV-18	C-type particles	A-type particles
Methylcholanthrene 200 µg subcutaneously	10/16	Tumour Spleen	5/9 3/9	2/8‡ 2/10	2/8 1/10
Methylcholanthrene 200 µg subcutaneously—non-tumour group	0/6§	Spleen	0/7	1/6	0/6
No treatment—age-matched controls from geriatric colony	0/8	Spleen	2/7	2/8¶	2/8

* MSV-18 was of intermediate sensitivity for the complement fixing gs antigen (see text).

† Number of tumours/number of surviving mice.

‡ C-type and A-type particles were in different tumours. The two tumours and one of the two spleens with C-type particles were complement fixing antigen positive.

§ The six surviving MC-inoculated mice without tumours were killed 148 days after inoculation.

|| These eight mice were killed after 18 months in captivity.

¶ These two spleens had both C-type and A-type particles but were complement fixing antigen negative.

Table 3 Spontaneous Tumours in Wild Mice

Type of tumour†	Tissue	CF gs antigen * MSV 21 and 25	C-type particles	A-type particles
Lymphoma	Lymph node tumour	4/4‡	4/4	0/4
Sarcoma	Spleen	8/8	3/5	1/5
	Tumour	3/4	0/5	1/5
Pulmonary adenoma	Spleen	1/5	0/4	1/4
	Tumour	4/4	0/3	1/3
	Spleen	1/4	0/2	0/2

* MSV-21 and MSV-25 were of greatest sensitivity for the murine C-type tumour virus gs antigen (see text).

† The lymphomas were lymphoblastic, nonthymic and primarily involved the spleen. The fibrosarcomas were located in the thigh, low back, face and liver. The pulmonary adenomas each occupied nearly one entire pulmonary lobe. The period under observation before these tumours occurred was 19–26 months.

‡ Number positive/number tested.

of six lymphomatous mice examined but not in the sarcomas or pulmonary adenomas. At the time of tumour harvest, antibodies against polyoma and the other indigenous viral antigens were absent from the sera of the seven mice so tested (lymphoma—4; sarcoma—1; pulmonary adenoma—2). Polyoma virus particles were not seen in any of the MC induced or spontaneous tumours.

Virus Particles and CF Antigen

In a recent preliminary survey of several tissues from freshly trapped untreated wild mice, C-type particles were seen in two of twenty livers, one of six kidneys, six of ten large intestinal mucosae and five of eight uterine endometria. In one embryo a few C-type particles were noted budding from nucleated erythrocytes in the hematopoietic liver. Intracisternal A-type particles were also seen on occasion in this untreated group in postnatal spleen, liver, intestine, skeletal muscle, breast, uterus, placenta and in embryonic liver and skeletal muscle. B-type particles were seen in normal lactating breast tissue from two of four pregnant mice examined. In general, the prevalence of complement fixing antigen in these tissues, using the most broadly reactive MSV rat serum pools (Nos. 21 and 25), was much greater than visualization of C-type particles and those tissues showing C-type particles were usually, although not

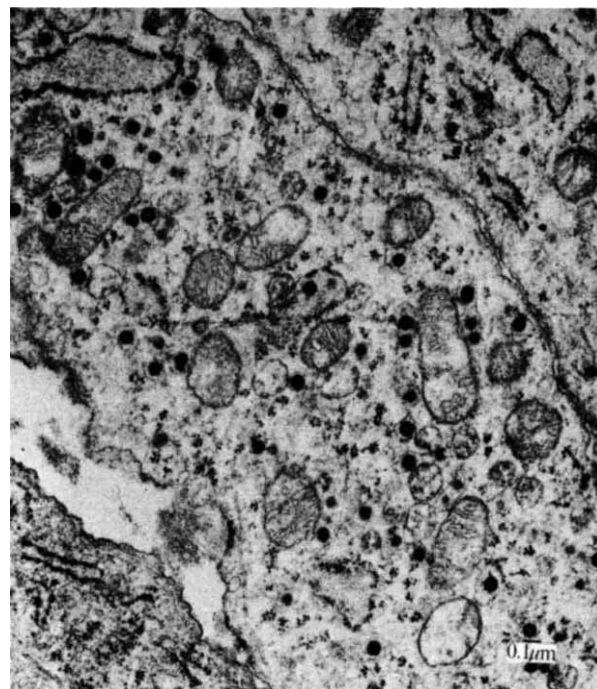


Fig. 2 Many 70-nm intracisternal A-type particles in the cytoplasm of an MC-induced sarcoma cell. (Uranyl acetate; $\times 22,800$.)

invariably, positive for this antigen (M. B. G., J. D. E., and R. J. H., unpublished observations).

Implications of the Findings

These findings demonstrate the presence of the C-type RNA tumour virus genome in tissues of feral wild house mice unexposed to laboratory infection or to manipulated breeding programmes. It seems clear that the inbreeding of laboratory mice has produced several strains in which the indigenous RNA tumour virus genome was more frequently expressed in terms of gs antigen, C-type particles and neoplasia than in the natural outbred species¹⁴; further, several inbred strains have been derived which have segregated for lesser expressions of this genome^{14,15}. The percentage of young adult wild mice showing detectable gs antigen in the MC-induced tumours and corresponding spleens and in untreated mouse spleens and other tissues depended on the sensitivity of the MSV rat serum pools used for the complement fixation procedure. With the most narrowly reactive MSV rat serum pool, the gs antigen could be detected in only a small percentage (14%) of spleens from untreated young adult mice or in their MC-induced tumours (10%). This corresponded closely to the prevalence of detectable C-type particles by electron microscopy in the spleens (14%) of untreated young adult wild mice and in the MC induced tumours (12%) and corresponding spleens (15%) of this age group. With the more broadly reactive MSV rat serum pool, capable of detecting a greater proportion of the gs antigenic complex, antigens were detected in larger quantities in MC-induced tumours (32%) and corresponding spleens (23%). The relatively low prevalence of detectable gs antigen in MC-induced tumours using the most sensitive MSV rat serum pool so far available is probably explained, in part, by hypothetical strong cellular repressor mechanisms for RNA tumour virus genome expression in mesenchymal tissues of wild house mice⁵. The comparatively low prevalence of gs antigen detection in spleens and MC-induced tumours (compared with laboratory strains) could also be partly attributed to some degradation of the gs antigen resulting from prolonged storage and repeat thawings of the tumour extracts before retesting with the more sensitive sera. The detection of occasional C-type particles by the electron microscope in both

tumour and spleen from only 12% of the mice with tumours also indicates that this latent genome is infrequently activated by MC to form visible virions. Infectious virus, however, has not yet been isolated from any of the MC-induced or spontaneous tumours, including those containing C-type particles, in spite of the use of the most effective virus isolation procedures^{7,16,17*}. The findings so far also indicate that in freshly trapped wild mice a few C-type particles may often be seen in various tissues. The extent to which natural activation of this latent murine RNA tumour virus genome to form gs antigen or C-type virions may depend on environmental carcinogens, indigenous DNA viruses such as polyoma, and/or ageing is under study.

In old mice there seems to be less suppression of viral genome activity as suggested (a) by the shorter latent period between MC injection and tumour formation, (b) by the higher prevalence and titres of gs antigen in tumours and spleens following MC inoculation, and (c) by higher incidences of spontaneous tumours in old compared with younger mice and the presence of gs antigen in almost all tumours of the older mice. Whitmire *et al.* also observed that 18 month old BALB/c mice treated with MC developed tumour and gs antigen much more often than younger mice treated with the same dosage of chemical (C. E. Whitmore, R. A. Salemo, L. S. Rabstein, R. J. H., and H. C. T., unpublished).

The absence of demonstrable antibodies to murine RNA tumour virus gs antigen (a 20% clarified extract of a Fisher rat MSV sarcoma) in all mice tested, including those with detectable gs antigen in tumour or spleen extracts, suggests that wild mice, like laboratory mice¹⁸, are immunologically tolerant to this ubiquitous antigen. This was also demonstrated by the absence of such antibodies in wild mice bearing MSV-induced tumours¹⁹. Such tolerance is perhaps best explained by vertical transmission and embryonic expression of the viral gs antigen which was demonstrated in the tissues of 16–18 day old wild mouse embryos trapped in Maryland^{5,19}. The absence of antibodies against polyoma antigen in mice bearing MC-induced and spontaneous tumours suggests that polyoma virus was not responsible for these tumours.

Virus Particles

The significance of the intracisternal A-type particles is unknown. These particles have been seen in several mouse tumours²⁰, including MC-induced sarcomas²¹ and carcinomas²². They have also been observed in muscle, liver and other tissues of embryos of a number of laboratory strains (M. L. Vernon, W. Lane, and R. J. H., unpublished observations) and in wild mouse embryos (M. B. G., R. W. R., and J. D. E., unpublished observations). They have not been shown to have biological activity²³, nor do they bear any known developmental or immunologic relationship to C-type particles^{21,23}. This point is borne out in the present study where A-type particles were found more often in gs antigen negative normal tissues and tumours while C-type particles were seen mainly in the tumours containing antigen.

The finding of B-type particles in the lactating breast tissue of two of four feral mice examined supports previous experimental evidence that wild mice carry the mammary tumour agent²⁴; but this particular RNA tumour virus has not been previously demonstrated in wild mice obtained directly from the feral state. Recent evidence²⁵ suggests that the B-type virus genome is similar to the C-type virus genome in being vertically inherited and largely repressed in most strains of mice by host cell genetic mechanisms.

It is doubtful that MC tumour induction *per se* could be attributed to superinfection of the tumour by infectious C-type

virus particles. Such particles were seldom found in the wild mice treated with MC and, as in laboratory mice, there seems to be no evidence that the virus is communicable in nature. Thus, an MC-induced immunological inability to neutralize infectious C-type virus is probably not an important mechanism in the pathogenesis of MC-induced tumours in wild mice. MC-induced suppression of immune surveillance mechanisms²⁶, however, may contribute to the proliferative growth of antigenically altered tumour cells. There is much evidence^{14,27} to support a role for C-type virus genome activation as a determinant of chemical or spontaneous oncogenesis in laboratory strains of mice. The selective activation of this genome to form gs antigen in the subcutaneous fibrosarcomas induced by MC in laboratory mice but not in adjacent mesenchymal tissue and muscle suggests that activation of this sarcoma oncogene in the tumour cells is often accompanied by activation of the gs antigen of the virogene^{5,14,27}. Our study suggests that in wild mice there is a similar causative relationship between chemical and spontaneous oncogenesis, ageing and activation of the latent C-type RNA tumour virus genome.

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* We have recent evidence from ³H-uridine particle labelling and positive XC cell tests¹⁷ suggesting the presence of infectious C-type leukaemia virus in several normal wild mouse embryo cultures after short term *in vitro* propagation (J. E. O., Hartley, and Freeman, unpublished observations).

LETTERS TO NATURE

PHYSICAL SCIENCES

Wobble Excitation by Earthquakes in Real Earth Models

THERE have been several accounts recently¹⁻⁴ describing connexions between polar motion and earthquakes. Because a simple correlation is of little significance unless a physical mechanism can be produced to explain it, the most important point discussed in these articles is the question of the efficacy of the mass displacements associated with major earthquakes in exciting the observed polar motion. We wish to report here calculations based on realistic Earth models. The elasticity theory of dislocations⁵, used to model earthquake displacement fields, has been generalized to take account of self-gravitation, the radial variation of elastic properties, density and gravity, initial hydrostatic stress and the presence of the liquid core. We have already reported initial results, giving the effect of such realistic Earth model displacement fields on the rotation⁶. Results of a later independent study have been reported by Dahlen⁷ and our complete study will be published elsewhere⁸.

The calculation of the polar shift and excited Chandler wobble (the free polar motion) is considerably simplified

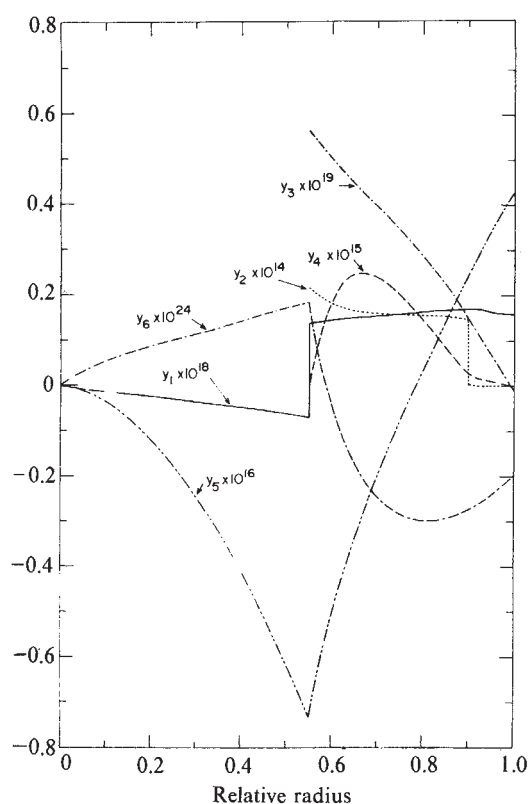


Fig. 1 Radial coefficients of one of the four spheroidal deformational modes affecting polar motion. The units are c.g.s. divided by the slip-fault area product (cm^3). This might be 10^{18} cm^3 for a major earthquake. Focal depth is 0.1 Earth radii.

because they depend only on four spheroidal displacement fields of second degree. This obviates the necessity of summing slowly convergent series to obtain the entire displacement field. Fig. 1 shows the radial coefficients of one of the displacement fields affecting polar motion. The six variables shown are the same as those used in free oscillation theory, representing radial displacement, change in normal stress, transverse displacement, transverse shear stress, decrease in gravitational potential and change in radial gravitational flux density, respectively.

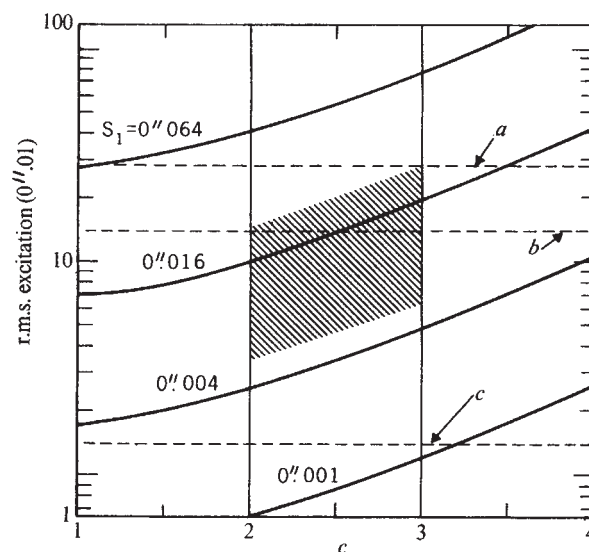


Fig. 2 Root mean square Chandler amplitude in terms of S_1 , the average polar shift for an earthquake of Richter magnitude 8.5, and the parameter c . The shaded area indicates the degree of uncertainty in the theoretically computed value. a , Double observed excitation; b , observed excitation; c , $1/10$ observed excitation.

For the first time in a static problem, the conditions at the core-mantle boundary have been treated without the assumption of the Adams and Williamson relation in the core. This relation takes the density profile to be that for a homogeneous material under compression in an adiabatic temperature field. Jeffreys and Vicente⁹ have shown that without the assumption of the Adams and Williamson relation in the core, application of the usual conditions at the core-mantle boundary leads to an insoluble problem. We have shown that in the static case, the conditions at the boundary must be modified; the modification allows the problem to be solved.

We compute that the Chile earthquake of 1960 shifted the pole 1.03 centiseconds of arc towards 269° E , and that the Alaska earthquake of 1964 shifted the pole 1.20 centiseconds of arc toward 51° E . These provide equal and opposite contributions to Chandler wobble excitation. We have used the fault parameters of Plafker and Savage¹⁰ for the Chile earthquake and those of Hastie and Savage¹¹ for the Alaska earthquake. Both earthquakes were of Richter magnitude $M=8.5$ but were rather unfavourably oriented for affecting polar motion. For shallow focus earthquakes of Richter

magnitude 8.5, the average polar shift is estimated to be between 0.5 and 2.5 centiseconds of arc. The dependence of average polar shift (S_1) on Richter magnitude is given by¹²

$$S_1 \times 10^{(M-8.5)c}$$

where c may have a value from 2.0 to 3.0. The range of the resulting r.m.s. Chandler wobble excitation is shown in Fig. 2.

Ben-Menahem and Israel² used a much simpler uniform Earth model and estimated that 30% of the Chandler wobble excitation may be attributed to seismic activity; this is evidently a lower limit. Our theory adequately accounts for the full excitation, but as most geophysical theories tend to be more precise than accurate we must await better measurements of both polar motion and earthquake deformation fields before the argument can finally be decided.

D. E. SMYLIE *

Department of Geophysics,
University of British Columbia,
Vancouver

L. MANSINHA *

Department of Geophysics,
University of Western Ontario,
London

* On leave at Code 550, Goddard Space Flight Center, Greenbelt, Maryland, 20771.

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Optical Variability of a Bright New Quasi-stellar Source

BROWNE¹ has identified the radio source 1215+30 of the Bologna B2 survey with a remarkably bright quasi-stellar source of magnitude approximately 14.5. We report here that its optical flux is variable by more than two magnitudes, with discernible brightness changes over time scales of hours, as well as secular trends on a time scale of years.

The light curve of B2 1215+30 (=ON325; J. D. Kraus, private communication) is given in Table 1. The data were obtained by visual estimates from fifty-eight Harvard plates taken between 1926 and 1950 with the f/5 16 inch Metcalf doublet. Table 1 lists (1) the plate number, (2) date, (3) exposure in minutes, (4) object brightness with respect to comparison stars A, B, C and D (identified in Table 2), (5) control brightness estimates of star B with respect to A and C, and (6) of star C with respect to B and D. The sequence A to D is in order of increasing brightness. The relative magnitudes are recorded in standard notation (for example, C9D denotes a magnitude brighter than C by 9/10 of the magnitude interval between C and D). Abbreviations used in Table 1 are bt, brighter than; ft, fainter than; nv, not visible, and s, slightly.

Table 2 gives the coordinates of the comparison stars in centimetres on the finding chart of Browne¹ taking the source as origin, with east to the left and north to the top of the chart. A transfer of magnitudes from nearby selected area 56

Table 1 Brightness of B2 1215+30

Plate	Date	Exp	QSS	B	C
21893	4-12-26	60	C9D	A3C	B7D
21910	5-4-26	61	B5C	A3C	flaw
22369	2-4-27	68	B8C	A2C	B7D
22395	2-27-27	62	B7C	A3C	B8D
22411	3-3-27	70	B5C	A3C	B6D
22579	6-18-27	62	C9D	ABnv	Bnv
23007	12-21-27	70	C	A3C	B6D
23083	1-20-28	68	C5D	A2C	B7D
23116	1-26-28	66	C9D	A2C	B7D
23203	2-21-28	63	sbt D	A2C	B6D
23213	2-24-28	108	D	A3C	B7D
23290	3-14-28	63	sbtD	A2C	B6D
23355	4-10-28	67	sbtD	Anv	B7D
23362	4-13-28	84	sbtD	Anv	B7D
23370	4-16-28	84	sbtD	A2C	B7D
23421	5-16-28	84	C2D	ABnv	Bnv
24124	3-9-29	69	ft B	Anv	—
24165	3-29-29	64	ft B	Anv	—
24174	4-3-29	58	ft B	Anv	—
24207	4-30-29	64	B	Anv	B7D
24221	5-7-29	77	B2C	A2C	B7D
24232	5-10-29	60	ft C	ABnv	Bnv
24699	1-4-30	77	B	Anv	B7D
24800	3-4-30	68	ft C	ABnv	Bnv
24831	3-21-30	83	B2C	A2C	B7D
24858	4-2-30	72	B2C	A2C	B7D
24890	4-22-30	74	ft C	ABnv	Bnv
24905	4-28-30	103	B2C	Anv	B7D
24928	5-16-30	64	ft C	ABnv	Bnv
24938	5-23-30	54	ft C	ABnv	Bnv
25297	1-13-31	96	B	Anv	B6D
25321	1-22-31	94	B	Anv	B6D
25327	1-24-31	97	B1C	Anv	B5D
25410	3-18-31	102	ft B	Anv	B7D
25440	4-11-31	92	B	Anv	B7D
25450	4-15-31	95	ft A	A2C	B7D
25808	12-15-31	43	ft C	ABnv	Bnv
25873	2-13-32	90	B	Anv	B5D
25944	3-12-32	114	B2C	Anv	B6D
26044	4-28-32	95	B1C	Anv	B6D
26363	2-24-33	45	B7C	A2C	B7D
26390	3-16-33	60	B4C	A3C	B6D
26395	3-23-33	60	B8C	A3C	B7D
26397	3-23-33	60	B4C	A3C	B7D
26401	3-24-33	45	B7C	A3C	B6D
26409	3-27-33	48	B8C	A3C	B6D
26412	3-27-33	60	B7C	A2C	B5D
26443	4-20-33	50	B8C	A3C	B7D
26445	4-20-33	50	B4C	A3C	B6D
26450	4-22-33	45	B6C	Anv	B7D
26452	4-22-33	45	B4C	A2C	B7D
31365	2-24-41	190	A	A3C	B6D
31433	4-17-41	70	A6B	A4C	B6D
31436	4-19-41	180	A4B	A3C	B8D
31437	4-20-41	120	A8B	A4C	B7D
31484	6-23-41	120	B2C	A3C	B8D
36940	11-6-50	13	ft C	ABnv	Bnv
36951	11-14-50	16	B7C	A4C	B7D

Table 2 Position of B2 1215+30

Star	A	B	C	D	QSS
Position (cm)	1.35 E 2.0 S	1.2 W 2.0 N	0.7 E 3.75 N	2.35 W 0.05 N	0.0 0.0

was made on three good quality 120 min exposure RH plates taken with the $f/7$, 3 inch Ross-Fecker camera. The range of variability was found to be $\Delta m_{pg} = 2.2$ or possibly slightly greater. The absolute range is less certain but is probably between 13.7 ± 0.2 and 15.8 ± 0.2 .

Table 1 shows that B2 1215+30 undergoes brightness fluctuations compared with the constant brightness comparisons. The fluctuations occur over months and years, and are even discernible over time scales of hours. For example, the change of 4/10 of the magnitude range B to C in the pairs of plates MC26395, 26397 and MC26443, 26445 is probably real in each case, and occurs over times of 0.10 and 0.14 days respectively. Further inspection of Table 1 shows that the QSS was systematically brighter in 1927/28 than at other times, and systematically fainter from 1929 to 1932, while in 1933 it seems to be systematically somewhat brighter again. The light curve is consistent with the interpretation that the optical output consists of long and short term variability which is known to be characteristic of compact galaxies and quasi-stellar radio sources.

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S. C. LUCCHETTI
P. D. USHER

*Astronomy Department,
Pennsylvania State University*

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Application of Laser Source Mass Spectroscopy to Analysis of Geological Material

THE preferred ion source for use in analytical mass spectrography is the spark source in its various forms such as the r.f. spark or the low voltage discharge, but some attention has been devoted in recent years to the possibility of using a focused laser beam to produce ions from the analytical sample^{1,2}. The advantage of using such a system lies in its ability to vaporize and ionize non-conducting samples.

Until now, emphasis has generally been placed on the fundamental principles involved, and the use of a laser source has been limited to very specific problems². This communication describes a successful attempt to use a pulsed neodymium laser as a source for the analysis of some elements in geological material.

One reason why lasers have previously been found unpromising as mass spectrographic sources is the slow pulse rates that were available, typically 1 pulse every 30 s. Furthermore

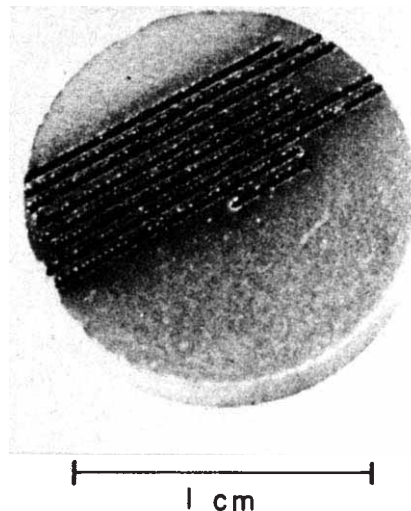


Fig. 2 Sample pellet after laser irradiation.

severe space-charge broadening effects occurred because often high power Q-switched laser pulses were used, especially in time-of-flight systems.

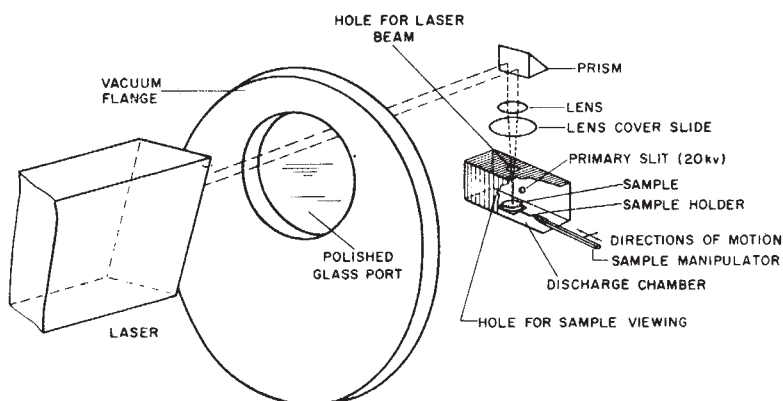
In the study described here, we have used a laser source with a pulse repetition rate of 6.25 s^{-1} in the non-Q-switched mode. Parameters of the laser radiation are given in Table 1. The laser was coupled to a double focusing mass spectrograph (Varian Mat SMIBF) to which some source modifications were made (Fig. 1).

Table 1 Parameters of Laser Radiation

Wavelength	10,600 Å
Mode	Conventional mode
Energy of burst	0.15 J
Total duration of burst	250 μs
Individual spike duration (½ width)	0.1–0.3 μs
Number of spikes per burst	~300
Mean burst power	~0.6 kW
Repetition rate	6.25 s^{-1}

The laser beam was focused on a spot on the sample (in the form of a pressed pellet or a rock chip) in front of the primary slit of the spectrograph. If allowed to, the successive laser pulses would drill into the sample, leading to a rapid diminution in plasma as the vaporization progressed. In the experiments described here, however, the sample was automatically moved laterally in order to present some unvaporized sample surface to each laser pulse. A constant scanning rate of 1 mm min^{-1} , or 3 μm per laser pulse, was found to be satisfactory. A typical sample pellet after irradiation, is shown in Fig. 2.

Fig. 1 Schematic diagram of modified source region of the mass spectrograph.



With the system described the total ion current was of the order 10^{-11} A. An ion-current of this magnitude permits analysis within a reasonably short exposure time. Inspection of the mass spectra revealed an extremely high sensitivity for the alkali metals. Furthermore, the spectral line shape was found to be excellent and much superior to that obtained using spark excitation. Rectilinear line profiles, which have been described as the ideal shape³, were obtained in all cases using laser excitation. This is presumed to be due to the formation of the plasma envelope at a constant position relative to the primary slit of the mass spectrograph, resulting in an ion beam geometry of constant solid angle.

A problem encountered in pressing pure rock powder such as G1 into a pellet was that the pellet did not bind satisfactorily. To overcome this problem, an admixture of 30% by weight of graphite (to act as binder) was made. We found that the presence of this graphite greatly increased the sensitivity for some elements. Variations in the quantity of graphite showed that in most cases less than 5% by weight of graphite was needed. It is thought that the presence of carbon in the laser plasma creates a reducing environment which assists in the fracture of metal-oxygen bonds.

Table 2 Limits of Detection (p.p.m.) for Typical Elements Found in Rocks*

Element	Without carbon	With carbon
Li	~0.05	~0.05
Na	≤0.05	~0.05
Mg	100	100
Si	20,000	200
K	≤0.01	≤0.01
Ca	20	20
Cr	100	25
Ti	50,000	200
Fe	200	20
Rb	<0.05	<0.1
Sr	50	15
Cs	≤0.1	~0.1

* The following elements were not detected, although present in the sample at greater than 1,000 p.p.m.: carbon, oxygen, fluorine, phosphorus, sulphur, and chlorine. The oxygen was seen as (M-O)⁺ complex only.

Assessed limits of detection for some elements in geological samples are given in Table 2. The specificity for the group one elements is noteworthy. We have made use of this characteristic to determine the lithium isotopic ratios in a number of terrestrial and extra-terrestrial samples which have reasonably low lithium levels.

The ⁷Li/⁶Li ratio obtained for W1 was 11.1 ± 0.3 in good agreement with the accepted value of 11.15. Values obtained for a number of South African stony meteorites were in the region of 14.0. Further studies of isotopic ratios are being undertaken.

Thus it has been shown that a medium pulse repetition rate laser can be successfully used as a routine source in mass spectrography where good sensitivity for the alkali metals is required. The excellent line shapes that can be obtained and the feasibility of *in situ* analysis of non-conducting samples are important advantages of the system described.

R. H. SCOTT
P. F. S. JACKSON
A. STRASHEIM

National Physical Research Laboratory,
Council for Scientific and Industrial Research,
Pretoria

Received May 17; revised July 30, 1971.

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Transformation of Hematite to Goethite in Soils

IN many parts of the world, especially in those that were not glaciated during the Pleistocene epoch, red hematitic soils that formed in a previous climate (different from the present one) still exist. In regions where the present climate is cooler and more humid than the previous one, the red colour seems to be disappearing and being replaced by more yellowish-brown colours that indicate the mineralogical transformation of hematite (α -Fe₂O₃) to goethite (α -FeOOH).

Near to the surface of profiles of these soils the proportion of red coloration decreases during these changes and the proportion of yellowish-brown colouring increases. A yellowish-brown rim forms on red mottles (in soils formed in the so-called mottled zones of former laterites exposed by truncation, for example) and this rim gradually grows inwards leaving only a red core that finally disappears. Hard nodules display a thin yellow skin covering a reddish hematitic interior. I have observed these features in areas as different as the North Island of New Zealand, South and Western Australia, Ceylon, Hong Kong and in south-eastern United States¹. Other people have observed them in Northern Japan² and Natal, South Africa³.

Both the reason for this transformation and its mechanism are closely connected to the change in the organic matter regime of the soil, which in turn reflects climatic change. A cooler and more humid climate causes a slower decomposition rate of the organic matter. In these conditions, increased amounts of organic compounds migrate into the soil and dissolve the hematite by reducing and/or complexing the iron. When this iron is reprecipitated (sometimes following oxidation) the newly formed oxide is usually goethite.

These observations are supported by laboratory experiments with synthetic iron oxides. In the pH range of most soils of humid climate areas (3.5 to 7.5) amorphous ferric hydroxide usually crystallizes *in vitro* to a mixture of goethite (through solution) and hematite (probably through internal dehydration). Hematite formation is stopped if the precipitation of the amorphous hydroxide is inhibited, or, in cases where it is formed, if it is prevented from dehydrating to hematite. This can happen when the iron released on weathering is complexed by organic compounds or when such compounds are adsorbed on the hydroxide. Recent experiments in this laboratory have shown that oxidation of ferric citrate yields pure goethite in a pH range where goethite and hematite are formed from ageing of amorphous ferric hydroxide⁵. Thus the higher the concentration of the organic compounds compared with the rate of Fe release from primary silicates, the greater the probability that goethite will be the dominating or only ferric oxide formed. The hematite-goethite ratio in soil therefore reflects the carbon regime and, in turn, the climate⁴.

The influence of organic matter is further supported by several observations. In cases where roots penetrate into old red soils, removal of iron normally occurs in the vicinity of the roots, forming a bleached zone around them. Some distance out from the root, the iron reprecipitates and forms a yellow rim that separates the bleached zone from the red soil matrix. Furthermore, a red soil which had come into close contact with a humus-rich surface soil by erosion and deposition showed a red→yellow transformation in the area of contact between the two layers. A similar hematite→goethite transformation through solution can also be observed in hydromorphic soils on red hematite sandstone (Bunter Sandstone, for example) in mid-Europe, where, under anaerobic conditions, hematite has gone into solution and the newly formed oxide is always goethite or lepidocrocite, but never hematite.

No change in climate has so far been definitely established at any of the localities where the colour change was observed. But from the striking similarities between the colour of soil profiles in these areas, and those in areas where such a climatic

change could be proved and the soils are definitely palaeosols (for example, Australia, N. Japan), a generalization seems to be justified. Therefore observations on these soils may have implications for palaeoclimatology.

Soils in which hematite (as well as goethite) is presently forming are well aerated and permeable, and slightly acid to calcareous. They occur under a warm subhumid to semiarid mediterranean or even arid climate (for example, Red Brown Earths of Australia⁶, Hamra soils of Israel, Red Desert Soils of California^{7,8}). This is in accord with the idea of a low ratio between supply of organic compounds and Fe release by weathering favouring hematite formation. Perhaps hematite is also forming in soils under moist tropical climatic conditions, where organic matter decomposes rapidly compared with a high rate of Fe release in acid conditions. This is, however, somewhat uncertain as many soils in these areas are probably non-recent and thus may have been influenced during their formation by other climates. Also at least some of the recent soils in these areas are brownish in colour rather than red.

U. SCHWERTMANN

*Institut für Bodenkunde,
Technische Universität München,
805 Freising-Weihenstephan*

Received March 18; revised June 7, 1971.

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In Support of a Physical Explanation of Ball Lightning

THE purpose of this communication is to present additional evidence in favour of the exclusion of explanations of ball lightning¹⁻⁶ in terms of, (a) the retinal afterimage hypothesis, (b) the intense point discharge hypothesis and (c) the burning material hypothesis. Altschuler⁷ lists these in his summary of possible explanations.

During a dinner on the evening of July 6, 1971, at Orselina, near Locarno, Switzerland, I observed a flash of lightning which struck an unidentified target near the roof of a building about 200 m away. An intense point discharge immediately followed the flash and was visible clearly at the target for 1 or 2 s. I immediately told the other people at the dinner (Professor E. H. Schröter and Dr E. Wiehr from Göttingen and Mr C. Kühne from Oberkochen) of my observation because they were not looking out of the window at the time.

This occurrence was unmistakably different from another lightning event, which I observed several years ago and which I consider to have been a rare event of actual ball lightning. At that time a thunderstorm accompanied by heavy rain took place in the area of Neustadt near Coburg, Germany. I was sitting with some other people (among them Mr C. Förster from Berlin) right behind a ground floor window so that I could look at the weather; the field of view out to the street was limited by the frame of the window. Suddenly, at a height of about 16 m above the ground and at a short range of about 24 m, I saw a spherical plasma ball coloured bright yellow-white. This object appeared to have a diameter of 50 to 100 cm. It moved vertically downwards with a speed of about 4 m s⁻¹, and its path ended in the top branches of a tree, at a height of approximately 9 m. On touching the tree the ball instantly disintegrated into eight to twelve smaller spheres. These were the same colour as the large one and each had a

diameter to 12 to 15 cm. They fell to the ground, guided by the outer contour of the tree, and moved vertically during the last few metres in the absence of branches. On reaching the ground (an asphalt roadway and a neighbouring footpath) the spheres instantly disappeared. There was no noise apart from that of the rain and no lightning associated with the primary plasma-like sphere. Three to five minutes afterwards, the same phenomenon occurred again in precisely the same way as before, indicating that the conditions needed to produce and guide the primary sphere were still maintained or re-established during the time that had elapsed. Immediately after the rain had stopped, I went out into the street to look for further evidence. There were circular patches of melted asphalt on the wet asphalt cover of the roadway which showed the interference colours of thin layers. Their diameters were each 12 to 15 cm and they obviously marked the impact areas of the smaller spheres. This event was described similarly by all the witnesses.

Because roadway asphalt in general contains B-80 bitumen—a thermoplast from which liquid components disintegrate at about 170° C—one may very roughly calculate the minimum amount of energy needed to produce the observed patches. Assuming that a water layer of thickness 0.5 mm at 20° C was evaporated and that an asphalt layer of thickness 1 mm was then heated to 170° C, and assuming a mean density of 1.0 g cm⁻³ and a mean specific heat $c_p = 0.46$ calorie g⁻¹ for B-80, one may easily calculate the energy density of the plasma spheres to be at least 1.9×10^7 J m⁻³.

A. WITTMANN

*Solar Physics Division,
Universitäts-Sternwarte,
Göttingen*

Received August 9, 1971.

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Proposal for a New High Precision Particle Detector

THE precision commonly quoted when measuring the position of a charged particle track is ± 0.3 mm for wire plane spark chambers¹, ± 0.4 mm for streamer chambers² and ± 0.7 mm for multiwire proportional chambers^{3,4}. With the onset of the new generation of synchrotrons there is a need for detectors with better resolution. This arises from the fact that the accelerated particles will receive only small deflexions in analysis.

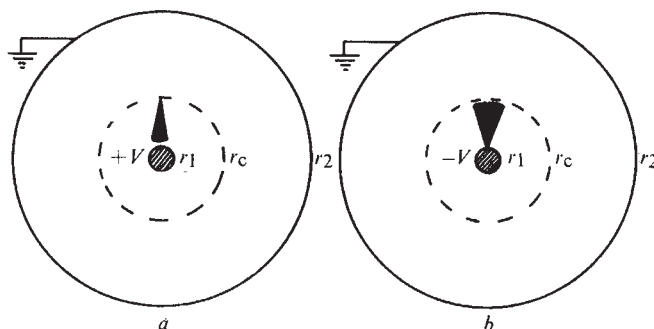


Fig. 1 a, Conventional proportional counter; b, outwardly directed avalanche chamber. Not to scale.

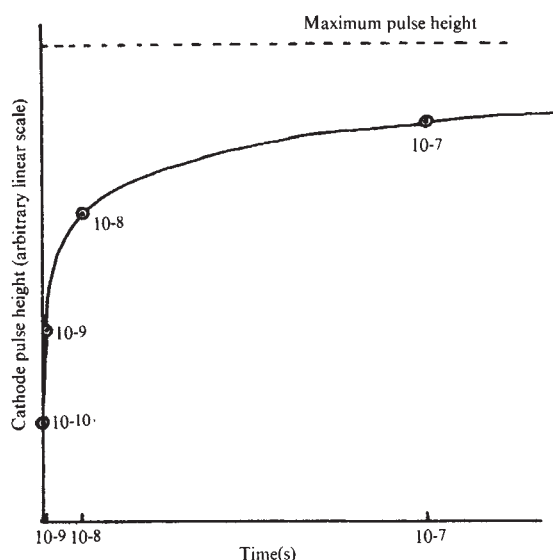


Fig. 2 Pulse formation in the outwardly directed avalanche chamber, calculated for the values mentioned in the text. The ions are collected at the wire cathode at $t_1 = 4 \cdot 10^{-9}$ s and the maximum pulse height is obtained at about $t_2 = 10^{-6}$ s when the electrons arrive at the cylindrical anode.

ing magnets. This article consists of a proposal for a detector that will provide the necessary increase in resolution.

The basic design of the proportional counter has remained unchanged since Rutherford and Geiger⁵. The pulse formation in such a counter has been discussed in detail by Wilkinson⁶. In a radial field where the electric field (E) is related to the applied potential (V) by

$$E = \frac{V}{r \ln r_2/r_1} \quad (1)$$

the electrons, from ion-pairs released by traversing charged particles, drift towards the anode wire (Fig. 1). On arrival at a critical radius

$$r_c = \frac{r_1 V}{V_c} \quad (2)$$

where V_c is the threshold voltage for ionization, secondary ionization starts. An avalanche results, the number of electrons roughly doubling in every mean ionizing path. Of the electrons falling on the wire, half are produced within only one mean path of the wire, and are collected practically instantaneously. The rise-time of the pulse, however, is governed by the drift of the positive ions away from the wire.

I shall consider the opposite design—in which the wire is the cathode. Any free electron at the surface of the wire will be accelerated radially and an outgoing avalanche will be formed (Fig. 1). The multiplication will continue until the critical radius is attained, after which the electrons will drift slowly to the cylindrical anode. The positive ions will accelerate towards the cathode where, on arrival, one assumes that they are merely neutralized. On completion of the avalanche at time $t=0$, one may assert that a shell of positive ions and electrons lies at the critical radius r_c . If the drift velocities be given by

$$\begin{aligned} u_+ &= \frac{dr_+}{dt} = -k_+ E \\ u_- &= \frac{dr_-}{dt} = k_- E \end{aligned} \quad (3)$$

where k_+ , k_- are the appropriate mobilities, one may show, using Green's theorem, that the growth of the cathode pulse with time is described by

$$P(t) = \frac{Ne}{C \ln r_2/r_1} \ln \left\{ \sqrt{\frac{2k_- Vt + r_c^2 \ln r_2/r_1}{-2k_+ Vt + r_c^2 \ln r_2/r_1}} \right\} \quad (4)$$

where C is the chamber capacity and Ne the charge in the avalanche. The positive ions will all be collected at time

$$t_1 = \frac{(r_c^2 - r_1^2) \ln r_2/r_1}{2k_+ V} \quad (5)$$

and the electrons at

$$t_2 = \frac{(r_c^2 - r_2^2) \ln r_2/r_1}{2k_- V} \quad (6)$$

Let the radius of the wire be 20 μm and that of the cylinder 1 cm. Suppose that the threshold voltage (V_c) is 1,200 V and the operating voltage 2,000 V. Then from equation (2), r_c is 33 μm . The avalanches will thus have a radial distance of 13 μm in which to develop. If the counter is filled with argon at atmospheric pressure, taking⁶ $k_+ = 2.5 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $k_- = 2,000 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, the pulse in Fig. 2 is obtained. Thus 22% of the maximum pulse is attained in 10^{-10} s and 65% in 10^{-8} s. The sharp rise means that a short detectable fast pulse may be obtained with a differentiation network.

If the "outwardly directed avalanche chamber" is to be a viable detector, particles must produce one free electron near the wire surface. The primary specific ionization in argon is about 27 ions/cm. Assuming that an electron produced inside a radius of 26.5 μm will result in a detectable pulse, the available distance is 13 μm —counting both sides of the wire. The chance of an ion-pair being produced in this distance is therefore $(13 \cdot 10^{-4}) (27) = 3.5\%$.

When a particle passes through the wire, a secondary electron may be emitted from the surface. This probability, δ , is thought to be independent of energy at high energies. Per-

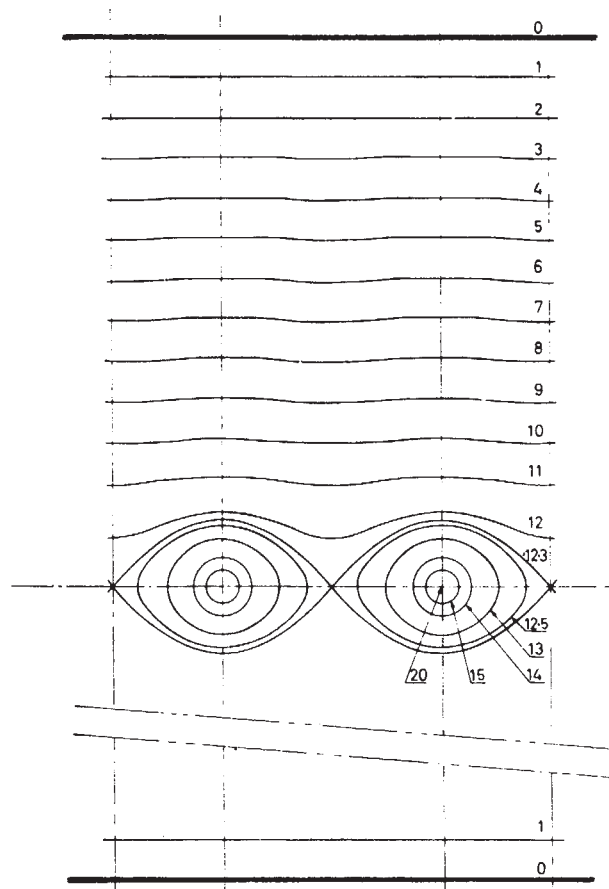


Fig. 3 The equipotentials surrounding the wires of a cathode wire-plane when placed between two plane anodes. It has been possible to take the figure from Charpak *et al.*^{3,4} as the electric field will have the same shape whether the wires be cathodes or anodes.

tinant values for δ are difficult to find. A value of $\delta=2.5\%$, however, has been given for 19.2 GeV/c protons traversing 5 μm aluminium foils⁷. Applying this to entrance and exit surfaces, the probability then becomes 5%. Bruining⁸ has mentioned that for 80 kV electrons, the probability increases by a factor 2 when the aluminium thickness increases from 3.6 to 9.4 μm . It seems reasonable therefore to use a factor 2 in applying the 5 μm value to 40 μm wire. Bruining has also commented on the effect of the density of the wire. For 300 kV electrons, the emission from tungsten (density 19.3 g cm⁻³) was 3.6 times that from aluminium (2.7 g cm⁻³). Thus if we use stainless steel wires coated with gold (density 19.3 g cm⁻³) our probability figure would become 36%—and more if the high field of the wire geometry enhances electron emission. Of course, these are rough estimates.

Quenching gas would be required to neutralize spurious ions produced by cosmic rays and non-intersecting particles. External quenching might also be necessary to cope with the action of the avalanche ions. Another possibility is to operate in a pure organic quencher—say, isobutane. Nevertheless, electron emission from the cathode might prove to be a difficult problem.

If successfully developed, the outwardly directed avalanche chamber might be used to assess the spatial distribution of particles in a beam. Alternatively, the wires might be arranged in a Charpak^{3,4} multiwire format in which the wires avalanche independently (Fig. 3). It is clear that the high resolution ($\sim 30 \mu\text{m}$) is bought at a cost of low efficiency—for example, a plane of 30 μm wires, spaced at 1 mm would be 97% transparent. Optimistically, one can envisage matrices comprised of hundreds of planes each with hundreds of wires.

I thank Drs Andreas Weitsch, Branko Bošnjaković, Robert Klanner and C. Schmied for discussing this work, and Professor P. Preiswerk and Dr Werner Kienzle for their hospitality at CERN, and also the Science Research Council for making my visit to CERN possible.

PETER RICE-EVANS

Physics Department,
Bedford College,
University of London,
Regent's Park, London NW1

Received August 24, 1970; revised May 15, 1971.

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BIOLOGICAL SCIENCES

Prostaglandin-induced Myometrial Activity inhibited by Progesterone

PROSTAGLANDINS (PGs) are being used increasingly as abortifacients although their precise mechanism of action is uncertain. One of the compounds frequently used is PGF_{2α}, which has been shown to stimulate the myometrium *in vitro*¹ and *in vivo*². Whether or not the direct action of this agent on the uterine muscle alone is responsible for the interruption of pregnancy

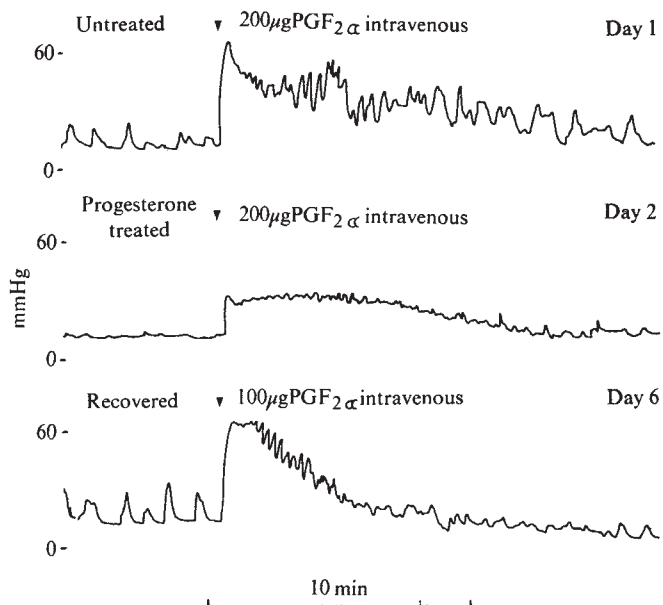


Fig. 1 Intra-uterine pressure recordings from a non-pregnant rabbit showing the response to an intravenous injection of prostaglandin PGF_{2α} before treatment with progesterone (day 1) during the progesterone effect (day 2) and after recovery from progesterone (day 6). NB. Treatment on day 6 was only 100 μg of PGF_{2α} whereas, on days 1 and 2, 200 μg of PGF_{2α} was used.

is not clear, but the long interval between infusion and abortion³ suggests that other mechanisms might be involved; for example, a luteolytic effect⁴⁻⁶. The prompt increase in uterine activity after treatment with prostaglandin, however, argues that the direct effect plays some part in the abortifacient action. Little is known of the influence of other myometrial regulatory agents such as the ovarian steroids on the uterine response to PGs. Accordingly we examined the influence of progesterone on myometrial responses to prostaglandin F_{2α} in the rabbit, a species in which progesterone is known to have a blocking action on the uterine muscle⁷.

Adult non-pregnant New Zealand White rabbits were equipped with intra-uterine recording balloons as described by Csapo, Takeda and Wood⁸ and intra-uterine pressure monitored via Statham pressure transducers and a Grass 7B polygraph. In twenty-three tests with four rabbits PGF_{2α} was administered by single intravenous injections of either 100 or 200 μg before, 12–14 h after treatment with 10 mg progesterone given subcutaneously when the inhibitory effect of this hormone on spontaneous myometrial pressure cycles was maximal, and also following recovery from the progesterone effect. In all animals the uterine response to both doses of PGF_{2α} was one of an immediate contracture. But, in the progesterone treated animals the amplitude, and with the 200 μg dose the duration, of the contracture was significantly less than in the untreated animals (Table 1). Following prostaglandin treatment rhythmic pressure cycles recurred before the resting pressure returned to the pre-treatment level in intact and recovered animals, but were reduced to a mere ripple (Fig. 1) in the progesterone treated rabbit. Injection of 200 μg of oleic acid intravenously as a control elicited no response from the uterus. In two further animals the effect of progesterone on the uterine response to the intravenous infusion of PGF_{2α} was examined. Whereas a single injection of 100–200 μg PGF_{2α} elicited a marked contracture of high amplitude, infusion of a similar quantity of the substance over a period of 30 min produced no elevation of the resting pressure but increased the frequency of pressure cycles by more than 100% (that is, mean frequency before treatment was 45.6 ± 13.0 s.e. pressure cycles/h, during infusion 94.8 ± 18.4 s.e./h (before against during $P < 0.1$) and after infusion 27.6 ± 7.0 s.e./h (during against after $P < 0.01$). After

Table 1 Myometrial Response to PGF_{2α} in Untreated, Progesterone-treated and Progesterone-recovered Rabbits (Four Animals)

	Treatment intravenous	Untreated and recovered ± s.e.	Progesterone treated ± s.e.	P
Amplitude of response (mm Hg)	100 µg PGF _{2α} 200 µg PGF _{2α}	37.8 ± 2.2 (8) 40.8 ± 3.7 (6)	22.4 ± 2.2 (3) 24.8 ± 3.3 (6)	< 0.01 < 0.001
P		> 0.1	> 0.1	
Duration of response (min)	100 µg PGF _{2α} 200 µg PGF _{2α}	9.5 ± 0.9 (8) 14.5 ± 1.1 (6)	9.7 ± 0.6 (3) 9.9 ± 1.1 (6)	> 0.1 < 0.025
P		< 0.025	> 0.1	

Figures in parentheses = number of tests.

treatment of the animals with 10 mg progesterone, this response was either totally abolished or so reduced as to be almost imperceptible, but returned on recovery from the progesterone block.

These results demonstrate that progesterone inhibits the myometrial response to PGF_{2α} in the rabbit. This might be due to its action in impairing the electro-coupling of cells⁷, or in reducing their reactivity by hyperpolarization of their cell membranes⁷, or both. Whatever the underlying cause it is clear that PGF_{2α} is a much less effective oxytocic agent in the presence of the progesterone-block. The significance of this in relation to its abortifacient action in women cannot be judged until the role of progesterone itself in maintaining human pregnancy is clarified. If progesterone is a major uterine regulatory agent in the human female, then the results of these experiments suggest that alternatives to a direct action on uterine muscle such as an interference with steroidogenesis should not be overlooked in seeking the mechanism by which prostaglandins induce abortion.

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D. G. PORTER

Department of Anatomy,

H. R. BEHRMAN

Department of Physiology,
Harvard Medical School,
25 Shattuck Street,
Boston,
Massachusetts 02115

Received March 3, 1971.

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Necessity for Restudying Nucleic Acid and Protein Synthesis in Mammalian Semen

It is generally accepted that mature spermatozoa cannot synthesize nucleic acids¹. The only enzyme involved in nucleic acid synthesis which has been identified in semen is polynucleotide phosphorylase², but this finding remains uncon-

firmed, and it has been suggested that the apparent enzyme activity resulted from interactions of other enzymes not involved in nucleic acid synthesis³.

We have reported the existence of a very potent compound in bull seminal plasma and sperm cell extracts which inhibited tRNA methylation^{4,5} by degrading the tRNA substrate into non-dialysable polynucleotide fragments⁶. Evidence strongly suggested that the active compound was not a ribonuclease, but a polysaccharide which formed an irreversible complex with the tRNA before degradation of the tRNA⁶. Data are presented here to demonstrate that very dilute amounts of seminal plasma or sperm cells are sufficient to hydrolyse all species of RNA. Consequently, the activity of exogenously added enzymes which synthesize RNA, or utilize it as a substrate or template, were undetectable in the presence of either of the semen components.

Semen from stud bulls collected over liquid nitrogen was supplied by the Eastern Artificial Insemination Corporation, Ithaca, New York. No antibiotics or preservatives were added to the final pooled sample. R17 viral RNA was provided by Dr James Eley. Ribosomal and transfer RNA samples were purchased from Miles Laboratories and General Biochemical Company, respectively. Bovine pancreatic ribonuclease and all nucleotides were purchased from the Sigma Chemical Company.

Seminal plasma was separated from sperm cells by centrifugation at 850g for 10 min. The sperm cells were washed twice with 0.25 M sucrose before use. Trichloroacetic acid was added slowly to 1:80 diluted samples of the semen components until solutions were made 7% with respect to the acid. After centrifugation, the supernatants were neutralized with NaOH and dialysed against water for 24 h before use. Final dilutions were made with distilled water. Preparing semen extracts in this manner inactivated an endogenous ribonuclease which preferentially hydrolyses double-stranded RNA (ref. 7 and B. S. and S. M. W., unpublished).

Percentage hydrolysis of the RNA samples was determined by incubating 0.1 mg of each species of RNA with various dilutions of seminal plasma or sperm cells in 1.5 ml. of 0.02 M Tris buffer (pH 7.8). After 30 min at 37° C, 0.2 ml. of 50% trichloroacetic acid was added to the assay mixtures to terminate the reactions. The mixtures were then kept at 4° C for 15 min before being centrifuged. The 260 nm absorbition of the supernatant solutions were then read to determine the amount of acid soluble products. Concurrent blanks were assayed with each individual concentration of seminal plasma and sperm cells.

Tables 1 and 2 show the percentage hydrolysis of the different species of RNA after being incubated with various dilutions of the semen components. Dilutions as low as 1:2,500 of seminal plasma, and 1:500 of sperm cells, were

Table 1 Hydrolysis (%) of RNA after Incubation with Varying Dilutions of Seminal Plasma

Dilution of seminal plasma	% Hydrolysis of 0.1 mg of				
	Viral RNA	tRNA	Polyuridylic acid	rRNA	
1:500	100	100	100	100	
1:1,000	100	100	100	100	
1:2,500	100	100	100	100	
1:5,000	100	93	93	81	
1:10,000	96	89	80	70	
1:25,000	92	84	70	60	
1:50,000	85	79	52	33	
1:100,000	44	40	20	9	
1:200,000	19	14	4	0	
1:400,000	10	10	0	0	
1:750,000	5	1	0	0	
1:1,000,000	1	0	0	0	

The preparation of the seminal plasma and the assay for RNA hydrolysis are described in detail in the text.

The RNA samples used were R17 viral RNA, *E. coli* tRNA, and *E. coli* rRNA.

Table 2 Hydrolysis (%) of RNA after Incubation with Varying Dilutions of Sperm Cells

Dilution of sperm cells	% Hydrolysis of 0.1 mg of			
	Viral RNA	tRNA	Polyuridylic acid	rRNA
1: 500	100	100	100	100
1: 1,000	100	100	96	89
1: 2,500	100	98	75	71
1: 5,000	85	79	49	35
1: 10,000	73	65	20	14
1: 25,000	68	50	4	3
1: 50,000	31	26	0	0
1: 100,000	13	12	0	0
1: 200,000	1	0	0	0

The preparation of the sperm cells and the assay for RNA hydrolysis are described in detail in the text.

The RNA samples used were R17 viral RNA, *E. coli* tRNA, and *E. coli* rRNA.

both able to hydrolyse totally 0.1 mg of tRNA, rRNA, polyuridylic acid or R17 viral RNA. There were no significant differences in the percentage hydrolysis of tRNA samples isolated from different sources.

Because of the degradation of RNA, these dilutions of the semen components completely masked the activity of exogenous enzymes which required RNA as a substrate, such as tRNA methylases⁵, rRNA methylases⁵ and valine tRNA aminoacylase⁸; and enzymes which synthesize RNA such as DNA-dependent RNA polymerase⁹, and uridine diphosphate polymerase¹⁰. These data were independent of the degree of activity of the exogenous enzyme preparation. Where applicable, 0.1 mg of RNA was employed as the substrate. Similar results depicting the masking of enzyme activity were obtained with concentrated or diluted samples of seminal plasma or sperm cells which were not treated with the trichloroacetic acid.

Bovine pancreatic ribonuclease activity was not affected by either of the semen components. This may be explained by the fact that the active compound in semen degrades tRNA into non-dialysable fragments⁵. It can therefore be assumed that these fragments, being non-dialysable, are large enough to serve as a substrate for the exogenous ribonuclease. The data in these studies indicate that unless the hydrolytic substance in semen is differentially inactivated, it will be impossible to detect (except for diesterases) any exogenous or endogenous enzyme activity in semen, whereby RNA is synthesized, or utilized as a substrate or template. Initial studies have demonstrated that periodate oxidation destroys the hydrolytic substance in very dilute semen without affecting endogenous nucleic acid polymerase activity. Experiments are now being conducted to determine the proper conditions to inactivate this compound in undiluted semen by periodate oxidation. When this is accomplished, it will be possible to determine whether mature spermatozoa possess the enzyme complement necessary to synthesize nucleic acids and proteins.

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BERTRUM SHEID
SUSAN M. WILSON

Department of Pharmacology,
State University of New York,
Downstate Medical Center,
Brooklyn, New York 11203

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Possible Role for Prostaglandin F_{2α} in Parturition in Sheep

PROSTAGLANDINS F_{2α} (PGF_{2α}), E₁ (PGE₁) and E₂ (PGE₂) have potent oxytocic properties that have led to their successful use in induction of labour and abortion in women¹. They have been found in tissues and biological fluids of women in spontaneous labour but it is not clear whether the release of prostaglandins has an important physiological role in the control of labour or whether it is a consequence of labour. Karim and Devlin² found PGE₁, PGE₂, PGF_{1α} and PGF_{2α} in amniotic fluid of women in labour although only PGE₁ was present in samples obtained before labour. The source of prostaglandins in amniotic fluid may be the decidua, which shows the same pattern of prostaglandins as amniotic fluid—PGE₁ before labour and PGE₁, PGE₂, PGF_{1α} and PGF_{2α} during labour². The decidua may also be the source of PGF_{2α} found in peripheral venous blood³.

In sheep, the mechanism of onset of labour seems to be controlled chiefly by corticosteroids secreted by the adrenals of the foetal lamb⁴. The length of pregnancy can be manipulated in this species by experimental procedures such as foetal hypophysectomy or foetal infusions of either adrenocorticotrophic hormone or corticosteroids that depress or augment foetal adrenal function. We have investigated the role of PGF_{2α} in premature parturition induced by infusion of a glucocorticoid into the foetal lamb.

Samples of maternal cotyledons, foetal cotyledons and myometrium were obtained by killing ewes with an overdose of phenobarbitone and gallamine and then rapidly excising the tissues. The control ewes were normal pregnant animals of known gestation length. In one experimental group, the foetuses had received an intra-carotid infusion of dexamethasone (1 mg/24 h) either for 24 h or until labour contractions were observed on continuous intra-amniotic pressure recordings (at 46–52 h). In another experimental group the foetuses were infused with dexamethasone for 52–57 h but the onset of labour was prevented by giving the ewe progesterone in oil 100 mg/12 h. Before and during corticosteroid-induced labour large samples of plasma (100 ml.) were obtained by plasmaphoresis from chronically implanted catheters in the maternal jugular (JV) and uterine veins (UV). The fresh samples were immediately frozen on dry ice and stored at –10° until analysed.

Weighed portions of tissue were homogenized in 4 volumes of ethanol at 4° C. The supernatant of the homogenates and the plasma samples were extracted with ethyl acetate at pH 3.0 after the addition of 200 nCi of tritiated PGF_{2α}. After methylation, the PGE and PGF compounds were separated by thin layer chromatography (TLC) using system M1 of Gréen and Samuelsson⁵. Authentic standards of methylated PGF_{2α} and PGE₁ were run beside the unknowns and were identified by spraying with 1% of iodine in methanol. *R_F* values (relative to the solvent front) of the methyl derivatives of PGF, PGE and PGA were respectively 0.42, 0.605 and 0.87. Areas of the TLC plates corresponding to the standards were removed and the extracts were further identified and purified by paper chromatography at room temperature using a mobile phase of ethylene dichloride/heptane 1:1 and a stationary phase of 70% glacial acetic acid. The area

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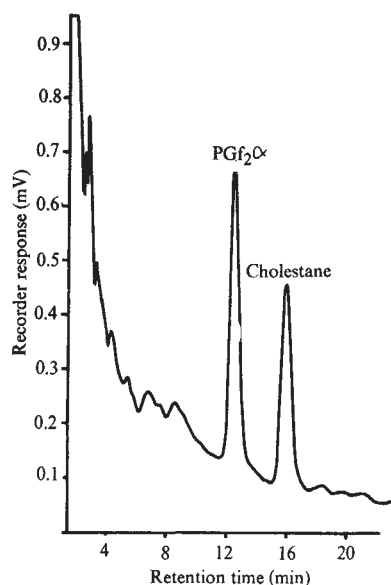


Fig. 1 Gas chromatographic assay of $\text{PGF}_{2\alpha}$ in 39 g of maternal cotyledons from sheep No. 0/102. Overall recovery during purification was 36%. One-twentieth of the trimethylsilyl ether derivative of the final extract was injected. Cholestane was added as an internal standard. The $\text{PGF}_{2\alpha}$ peak corresponds to 190 ng of authentic material. Column conditions: 1.8 m $\times$ 4 mm glass column, 2.03% SE 30 on 80–100 mesh 'Diaport S'. The column temperature was 225°C; injection port temperature was 260°C. Nitrogen flow rate was 25 ml./min.

corresponding to $\text{PGF}_{2\alpha}$ was identified by radiochromatogram scanning. The R_F values of PGF , PGE and PGB methyl ethers were respectively 0.52, 0.64 and 0.73. The trimethylsilyl ether derivatives were quantitated by gas chromatography (GLC) using conditions described in Fig. 1 and were also applied to a column packed with 3.8% 'Hi-Eff 3A' on 80–100 mesh 'Diaport S' at a column temperature of 218°C. Cholestane was used as an internal standard for PGF assays. Retention times relative to cholestane for authentic $\text{PGF}_{1\alpha}$ and $\text{PGF}_{2\alpha}$ were respectively 0.78 and 0.84 on the SE 30 column and 0.45 and 0.45 on the succinate column. PGE compounds eluted from TLC plates were converted to PGB compounds by treatment with NaOH and were then subjected to paper chromatography, acetylation and GLC. The internal standard used for GLC of PGB compounds was 20 β -acetoxyprogesterone and the relative retention time of authentic PGB_1 and PGB_2 on an SE 30 column was 0.72. Peaks with the same relative retention time as authentic materials on both SE 30 and succinate columns were measured and the amounts of prostaglandin in the samples were determined from a standard curve. The lower limits of detection of both PGF and PGE compounds were 100 ng/20 g tissue sample and 200 ng/100 ml. plasma sample. Procedural losses were estimated from the recovery of radioactivity after silylation.

$\text{PGF}_{1\alpha}$, PGE_1 and PGE_2 were not detected in any of the tissue or plasma samples. $\text{PGF}_{2\alpha}$ was not found in any of the JV samples or in UV samples taken before labour but was present in UV plasma during labour in nine of ten ewes (Table 1).

The concentration of $\text{PGF}_{2\alpha}$ in maternal cotyledons was raised above the levels found in two control animals after dexamethasone had been infused into the foetus for 24 h, but the concentration in myometrium was unchanged (Table 2). In labour, a further increase in concentration in maternal cotyledons was found and, in addition, the concentration in the myometrium was increased. Similarly increased concentrations of $\text{PGF}_{2\alpha}$ were also found in maternal cotyledons and myometrium of ewes that would have been in labour had they not received progesterone. The concentration of $\text{PGF}_{2\alpha}$ in foetal cotyledons was relatively low in all samples.

The results show that an increase in concentration of $\text{PGF}_{2\alpha}$ in maternal cotyledons precedes the onset of induced, premature labour by at least 24 h. Furthermore, an increase in concentration in both maternal cotyledons and in myometrium occurs not only in labour but also when uterine activity is suppressed by large doses of progesterone, indicating that the appearance of $\text{PGF}_{2\alpha}$ is not a consequence of labour. These observations are consistent with a causal relationship between $\text{PGF}_{2\alpha}$ and the onset of labour in sheep.

Table 1 Concentration of $\text{PGF}_{2\alpha}$ in Uterine Venous Blood before and during Premature Labour induced by Infusion of Dexamethasone into Foetal Lambs

Sheep No.	Length of gestation (days)	$\text{PGF}_{2\alpha}$ concentration (ng/ml.) Before labour	In labour
0/6	110	—*	31
0/39	111	—	11
0/36	117	—	5.4
9/104	120	—	—
9/82	126	—	7
9/107	127	—	37
0/86	131	—	35
0/64	138	—	2.4
0/61	140	—	29
0/85	140	—	3.5

* Not detected (< 2 ng/ml.).

Table 2 Concentration of $\text{PGF}_{2\alpha}$ (ng/g wet tissue) in Maternal Cotyledons, Foetal Cotyledons and Myometrium before and during Premature Parturition induced by Infusion of Dexamethasone into Foetal Lambs

Sheep No.	Gestational age (days)	Maternal cotyledon	Myometrium	Foetal cotyledon
Untreated				
0/92	126	82	70	47
0/93	140	164	88	122
After 24 h dexamethasone				
0/108	128	382	99	101
0/102	130	315	97	86
After about 48 h dexamethasone (in labour)				
0/100	128	543	490	77
0/101	128	470	154	—
0/113	130	633	257	—
0/50	140	875	417	100
0/62	140	—*	515	114
After about 48 h dexamethasone (labour suppressed by progesterone)				
A254	126	329	122	110
0/107	127	723	236	33
0/103	134	275	306	—

* Sample lost during purification.

The plasma concentration of progesterone decreases sharply before parturition at term⁶ and also before premature parturition induced by administration of corticosteroids or ACTH to the foetus⁴. Although it is likely that withdrawal of the inhibitory effects or progesterone on uterine contractility plays a part in the physiological mechanisms regulating labour in sheep, there is doubt that it is the major mechanism since relatively large doses of progesterone fail to prevent labour^{4,7}. Progesterone withdrawal may have a facilitatory role in parturition, secondary to a mechanism that is dependent on prostaglandin release. The recent demonstration that the uterine luteolytic agent is likely to be $\text{PGF}_{2\alpha}$ (refs. 8, 9) raises the possibility that $\text{PGF}_{2\alpha}$ could serve a dual role in relation to parturition, being luteolytic in species in which luteolysis immediately precedes parturition and being oxytocic in species in which pregnancy is maintained by placental hormones.

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G. C. LIGGINS
SUSAN GRIEVES

Postgraduate School of Obstetrics and
Gynaecology,
University of Auckland

Received March 15; revised April 30, 1971.

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Purity of Synthetic Peptide Preparations

WE strongly endorse Vane's comments¹ on the pitfalls associated with the use of synthetic peptides in biological studies. It is particularly misleading to express results in terms of weight without evidence of homogeneity, because apart from error peptides (which may not be inert), account is seldom taken of salt and solvent content.

The impact of these problems in the field of radioimmunoassay was discussed at some length^{2,3} at the recent workshop meeting in Edinburgh. In the case of one commercial synthetic angiotensin already widely used in research, evidence has been recorded of varying stability between batches⁴ and apparent variations in the peptide content of different batches⁵. Furthermore, extending Vane's comments on the chemical purity of these peptides, it may not be generally appreciated that rearrangement of the aspartyl residue in angiotensin can occur in solution even at neutral pH; the biological activity of the β aspartyl isomer in the rat pressor assay is some 150% that of the α form⁶. The activity of successive batches of peptide could thus differ according to the degree of contamination by the β isomer. If, however, as suggested¹, manufacturers provided comprehensive analytical data for each batch of synthetic peptide, the user would be aware of this possibility.

When precise quantification is needed, the practice of describing results in terms of apparent peptide content is misleading and could be avoided by assay in terms of a suitably characterized standard preparation. Such a preparation of angiotensin II (asp¹ ileu⁵ angiotensin II) has been chemically characterized and its stability studied; this preparation (coded 70/302) and also a human renin preparation (coded 68/356) are now available from this division to research workers for the purpose of standardization.

In due course a preparation of human type angiotensin I⁷ will also be provided. It is hoped that use of these will make possible valid quantitative comparison of results in the renin/angiotensin field.

We would emphasize that these general comments apply to many of the synthetic peptide hormones used quantitatively in research which include oxytocin, vasopressins, angiotensins

I and II, bradykinin, corticotrophins, human calcitonin M and secretin.

D. R. BANGHAM
D. H. CALAM
J. A. PARSONS
C. J. ROBINSON

Division of Biological Standards,
National Institute for Medical Research,
London NW7

Received May 14, 1971.

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Role of Reduced TPN in the Response to Interstitial Cell Stimulating Hormone

DISCUSSING the mechanism of action of interstitial cell stimulating hormone (ICSH), also called luteinizing hormone, on the ovary, Flint and Denton¹ concluded that the "primary steroidogenic response" of rat luteal tissue to ICSH involves no change in the cytoplasmic concentration of TPNH (NADPH). This argument was based on two observations: (i) glucose added *in vitro* to luteal tissue increased TPNH/TPN⁺ but not steroid synthesis and (ii) ICSH *in vivo* increased steroid synthesis but not TPNH/TPN⁺. Steroid synthesis was observed to increase by 0.19 μ mol/g/h after injection of ICSH. The interpretation proposed reveals a fundamental misunderstanding of the significance of steady state values of TPNH/TPN⁺ which this letter attempts to clarify.

In steady state conditions the concentration of luteal TPNH (or the ratio TPNH/TPN⁺) at any one time is the outcome of a balance between production and destruction of TPNH (effectively between the relative rates of oxidation and reduction of the pyridine nucleotide). Clearly, constancy of steady state value of TPNH (or TPNH/TPN⁺) does not necessarily mean that the rate of reduction of TPN⁺ remains constant; the only valid conclusion is that the balance between oxidation and reduction has not changed. If the rate of reduction of TPN⁺ increased two-fold, steady state values of TPNH/TPN⁺ would not alter, if at the same time, oxidation of TPNH also increased two-fold.

Steroid-forming organs do not maintain high levels of stored biosynthetic intermediates beyond cholesterol, so that trophic stimulation depletes such stores of cholesterol (refs. 3-5 and unpublished observations). The synthesis of 1 mol of gestagen (progesterone and 20 α -dihydroprogesterone) requires at least 2 ml. of TPNH to cleave the side chain of cholesterol². It follows that the administration of ICSH has resulted in the formation of at least 2 $\times$ 0.19 μ mol TPNH/g/h above levels in control tissue (no ICSH). The fact that steady state levels of TPNH/TPN⁺ are unchanged should not obscure this. At least three possible explanations for increased formation of TPNH without change in TPNH/TPN⁺ following administration of ICSH can be suggested. (i) ICSH increases the rate of production of TPNH to the level required to accelerate steroid production by 0.19 μ mol/g/h; the additional TPNH is oxidized

in the process of producing the steroids at an accelerated rate; the ratio TPNH/TPN^+ remains unaltered. (ii) ICSH specifically decreases oxidation of TPNH by some means not connected with steroidogenesis and this decrease equals the increase in the rate of utilization of TPNH for accelerated steroidogenesis. The equality of these two rates may reflect some controlled coupling between the two processes concerned. (iii) ICSH stimulates steroid synthesis by a mechanism not involving increased production of TPNH . Steroid synthesis could in turn generate reduction of TPN^+ "as it goes", by some unknown mechanism; this coupling between steroid synthesis and the reduction of TPN^+ would preserve steady state levels of TPNH . If the experiments reported were not carried out in steady state conditions, the data are more difficult to interpret and thus less informative.

The same considerations apply to interpretation of steady state concentrations of hormones in plasma which do not necessarily reflect the rate of production of the hormones concerned; a certain concentration can be maintained by low production with slow metabolic clearance or by high production with rapid metabolic clearance⁶.

The first experimental observation of Flint and Denton¹ tells us that glucose increases TPNH/TPN^+ but not steroid synthesis. As they point out, the TPNH required for side chain cleavage of cholesterol must act within mitochondria. Ratios of TPNH/TPN^+ for the whole cell tell us nothing about mitochondrial TPNH . There is no reason to believe that enhanced glucose-6- PO_4 dehydrogenase activity will automatically generate intramitochondrial TPNH ; *prima facie* this possibility seems quite remote. The central dilemma of the Haynes hypothesis to account for the action of ACTH on adrenal steroid synthesis⁷ is the difficulty of explaining how high levels of intramitochondrial TPNH can result from increase in cytoplasmic TPNH/TPN^+ . Simpson and Estabrook⁸ suggested that malate may serve to "shuttle" reducing equivalents from cytoplasm to mitochondria. If this is so there are two possible consequences.

(i) If ICSH acts by providing TPNH for side chain cleavage of cholesterol, total cell levels of TPNH need not change after administration of ICSH, because intramitochondrial TPNH would increase at the expense of cytoplasmic TPNH . The additional reducing equivalents would be used within the mitochondria as fast as they were transported there from the cytoplasm.

(ii) Increasing cytoplasmic TPNH/TPN^+ by addition of glucose or by enhanced activity of glucose-6-phosphate dehydrogenase, will not *per se* increase steroidogenesis, unless the production or transport of malate is also increased to convey the extra reducing equivalents into mitochondria. The two observations of Flint and Denton can therefore be readily explained, even if ICSH stimulates steroid synthesis only by increasing reduction of TPN^+ .

The misunderstanding revealed in the proposed interpretation of these data arises from the idea that if stimulation of steroid synthesis results from increase in TPNH/TPN^+ , the cell establishes this stimulation by first developing a head of reductive energy followed by increased output of steroids. It could equally well be that steroid synthesis is poised ready for any increase in TPNH ; reduced pyridine nucleotide would be formed more rapidly but would not accumulate. One important result of a systems approach to the control of steroid synthesis has been the demonstration of rapid return of steroidogenesis to control levels when trophic stimulation is withdrawn⁹. The sort of lag expected from the "running down" of accumulated high levels of TPNH is not observed.

Even if the cell sets up a high ratio of TPNH/TPN^+ which then drives steroid synthesis at an accelerated rate, it seems likely that the high levels of TPNH would soon be consumed. High levels of TPNH may not persist for 1 h after injection of ICSH.

The importance of increased production of TPNH in explaining the mechanism of action of ICSH will only become clear

when we know more about the intracellular compartmentalization of pyridine nucleotides and when we can monitor continuously the redox state of TPN . TPNH may or may not be the fundamental mediator or the action of ICSH, but negative data on steady state values of TPNH/TPN^+ at a single time interval do not facilitate evaluation of this concept.

PETER F. HALL

Department of Biochemistry,
University of Melbourne,
Parkville,
Victoria

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Chromosomes of Cancer Cells

THE reported¹ alterations in the malignant potential of a virus transformed mouse fibroblast cell line and its relationship to variations in the chromosome number seems to have a parallel in the human cervix. Our studies of the chromosome patterns seen in pre-invasive and invasive carcinomas of the uterine cervix suggest that these patterns may be an indicator of the invasive potential of the pre-invasive lesion². We have proposed a theory in which we contend that the tetraploid and hypertetraploid pre-invasive lesions are less likely to develop into invasive carcinoma and that a potentially invasive lesion will have a peri-diploid component in its cytogenetic profile³.

In human cervical cancer the differences in chromosome number may be related to the nuclear size of the component cells. Small, mixed and large cell lesions occur in both types of neoplasm². It seems from our data that large cell pre-invasive lesions are cytogenetically distinct from large cell invasive carcinomas, the latter having a mean count of seventy-one compared with seventy-nine for pre-invasive cancer.

In both pre-invasive and invasive carcinoma of the cervix there is a wide distribution of chromosome number which cannot be described fully by the median⁴. We devised a statistical model to describe the total chromosome number distribution within individual lesions³. The use of the mean and variance to describe the component populations of the individual lesions shows a change in pattern which seems to occur between the pre-invasive lesions and invasive lesions. Pre-invasive lesions with chromosome counts in the tetraploid, quintaploid and hexaploid ranges were not represented in the invasive cancers and we suggested that a peri-diploid or peritriploid dominance was required before invasion could take place³. Few examples of progression from pre-invasive to invasive cancer have been observed in human material, but an example of progression to micro-invasion with the appearance of a peri-diploid chromosome complement has been reported⁵. Present studies in this laboratory also seem to indicate that the sensitivity of invasive carcinoma of the cervix to radiotherapy may be related to variations in the chromosome constitution.

Overall, the comparison of the patterns in pre-invasive and invasive cancer of the cervix suggests that hypertetraploidy is associated with a low degree of malignancy. It seems possible, therefore, that these previously extraordinary variations in

chromosome constitution are related to the progression of the individual neoplasm, but only when the entire chromosome complement is considered. The changes in the chromosome pattern which have been reported in a virus transformed cell line seem to have a clinical counterpart, particularly when the total cytogenetic data for cervical neoplasia are considered. It is also of interest that a virus (Herpes type II) has been implicated as an oncogenic agent in the human cervix⁶.

J. A. KIRKLAND
M. A. STANLEY

Department of Obstetrics and Gynaecology,
Queen Elizabeth Hospital,
Woodville,
South Australia

Received March 16, 1971.

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Collection of Lymph from Kidneys Homotransplanted in Man: Cell Transformation *in vivo*

ALTHOUGH there have been careful studies¹⁻³ of the successive steps of lymphocyte sensitization by histocompatibility antigens *in vitro*, it has not yet been demonstrated directly that the same cell transformation takes place *in vivo* after a human organ allograft.

The practical problem is to devise a method of collecting a representative sample of lymph from such a transplanted organ. The lymph formed in the merino sheep kidney is drained entirely by a small number of hilar lymphatic ducts, so that cannulating one of these vessels and tying off the others allows the collection of the entire renal lymph⁴. In most other animals, including man, this is not however possible, because the renal lymphatic ducts are dispersed and not concentrated in the hilum. We have, nevertheless, succeeded in cannulating one of the renal lymphatic ducts of kidneys homotransplanted in dogs and in man. This communication concerns our attempts in man.

One way to make visible the human lymphatic ducts is to inject 1 ml. of Evans blue into the renal cortex. Three minutes later, some lymph ducts are seen on the exterior aspects of renal vessels. A ligature of vascular silk can be tied around this duct to serve as a label. Lymphatic ducts are extremely difficult to see in the ischaemic kidney, but we find they become clearly visible 10 or 15 min after transplantation. A polyethylene cannula (interior diameter 0.35 mm) filled with a saline solution of heparin can then be introduced into the duct and fixed tightly with a vascular nylon ligature. The catheter is led out through the abdominal incision.

In four recipients of kidney allografts, renal lymph collection was possible for 7, 5, 10 and 8 days after transplantation respectively. Lymphocyte outflow was measured daily. A differential count of normal lymphocytes and other cells, if any were present, was performed. Cells were studied by light and electron microscope. Tritiated thymidine incorporation was measured daily in the lymph and every second day in the blood with a scintillation counter, using 1 μ Ci/10⁶ lymphocytes. Cellular radioautographies were performed daily.

The results in case 1 are shown in Fig. 1. This 29 yr old patient was given a kidney transplant taken from a cadaver

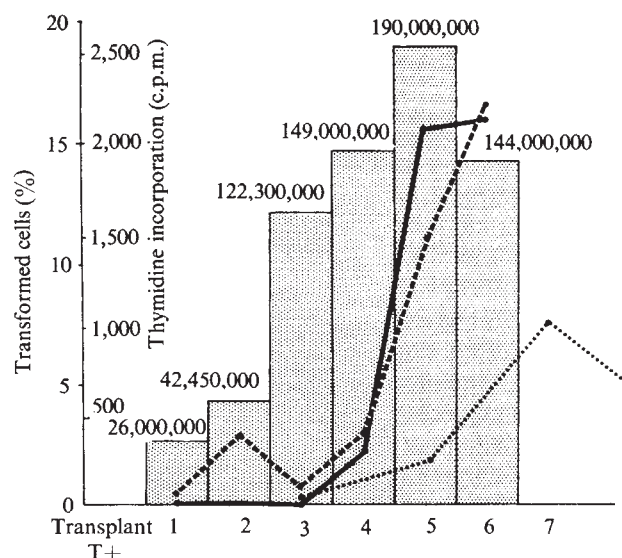


Fig. 1 Lymphocyte flow, percentage of lymph cell transformation and tritiated thymidine incorporation in lymph and in blood, following lymphatic duct drainage of an allografted kidney in man (case 1). Shaded sections, lymphocyte flow (cells/24 h); ● - - ●, thymidine incorporation in lymph; ●—●, percentage of transformed cells; ● · · · ●, thymidine incorporation in blood.

146 min after death. Renal ischaemia resulted in a 6 day oliguric renal failure followed by a progressive recovery. The patient received 300 mg of azathioprine, 5 ml. of anti-lymphocyte serum and 24 mg of betamethasone phosphate per day. The collected lymph contained 96–99% of small lymphocytes during the first 5 days. The total lymphocyte flow was successively 26 million, 42 million, 122 million, 149 million, 190 million and 144 million cells per 24 h. On days 5 and 6 there was a sudden appearance of transformed cells identical to the immunoblasts observed in mixed lymphocyte cultures and reaching 16% of the total cells. At the same time, the tritiated thymidine incorporation measurement rose from 91 c.p.m. on day 3 to 2,258 c.p.m. on day 6. Radioautographies showed a similar elevation of labelled cells. Tritiated thymidine incorporation into blood leucocytes also increased, but more slowly than in the lymph; after a peak on day 8 it decreased regularly. Coagulation stopped the lymph flow on day 7 and the catheter was removed. Case 2, in which the transplanted kidney was also taken from a cadaver, gave results similar to those observed in case 1, but the lymph flow ceased on day 5.

In cases 3 and 4 the kidney was taken from a living related donor. In case 3 (transplantation between siblings) a marked *in vitro* cell transformation (23% transformed cells) had been found in the mixed lymphocyte culture of donor and recipient cells before transplantation; the *in vivo* examination of the renal graft lymph showed essentially similar results to those found in case 1. In case 4 (parent to child transplantation) the mixed lymphocyte culture had shown no stimulation (1.6% transformed cells) and no cell transformation was found *in vivo* in the allograft lymph; neither was there any increase in thymidine incorporation. The clinical course in the latter case was remarkably uneventful.

These *in vivo* findings reinforce the suggestion that mixed lymphocyte culture may be considered "a model for allograft rejection"⁵. The similarity between the cell transformation taking place in the recipient of a human renal allograft and the cell transformation found in the *in vitro* mixed lymphocyte culture is striking. This has practical as well as theoretical importance because it suggests the immunological reaction may be studied and followed for several days in patients receiving a kidney allograft by using a daily collection of the graft lymph.

This may provide a new tool for recognizing and measuring the recipient sensitization, even in cases where the clinical signs of "rejection" are lacking or ambiguous. It also opens a new field for the *in vivo* collection and study of sensitized cells in human organ transplantation.

J. HAMBURGER
A. DIMITRIU
L. BANKIR
M. DEBRAY-SACHS
J. AUVERT

Hôpital Necker,
Paris XV⁰

Received October 22, 1970.

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Effect of Thalidomide on Human Embryonic Tissues

We wish to report results which can be used in an attempt to define the level of action of thalidomide on human embryonic tissues *in vitro*. Because it is difficult to obtain human embryonic tissues in quantities sufficient for biochemical measurements, these results are based on the morphology and histology of cultured human tissues.

The starting point for these studies was the observation¹ that the mesonephros of 2-3 day old chick embryos undergo chondrogenesis if cultured on nutrient agar, but not if it is wrapped in chorioallantoic membrane (compare ref. 2). The chondrogenic potential of the mesonephros adjacent to the appendages was greater than that of the intervening region². Thus chondrogenesis seemed to be related to girdle or appendage cartilage. Indeed, chondrogenesis is enhanced in 2-3 day limb buds cultured in association with the adjacent mesonephros, but not in buds from older embryos³.

An investigation of the effects of thalidomide on mesonephros-limb bud chondrogenesis in cultured chick tissues has shown that thalidomide inhibited mesonephric chondrogenesis, whereas 'Doriden' (a non-teratogenic analogue of thalidomide) had no effect. The inhibitory effect was clearly on the mesonephric tissue, not the limb bud tissue⁴.

Like most teratogenic agents, thalidomide has its greatest effect during a restricted period of development, with the human limb this occurs during the second month of pregnancy⁴.

We obtained human embryos from the Boije Clinic, Helsinki, and dissected out somites from the posterior limb region; presumptive girdle tissue from the same region; ventral spinal cord and notochord; limb mesenchyme, and the nephric tissue complex (mesonephros, metanephric bud and metanephric mesenchyme). These were cultured on nutrient agar (1% agar containing Simm's balanced salt solution (SBSS), foetal calf serum (FCS) and Coon's modification of Ham's F12 (F12X)), in the ratios 2:2:1. Foetal calf serum and F12X were obtained from Grand Island Biological Co. The liquid feeding medium used was SBSS:FCS:F12X, (2:2:1) with no supplement, with thalidomide (obtained from W. S. Merrell before court litigation began), or with 'Doriden' (Ciba).

Thalidomide and 'Doriden' were used in a concentration of 0.8 mg/100 ml. The nutrient medium was too dense to

allow spectrophotometric measurements of the thalidomide solution to check the concentration after the culture period. The half life of thalidomide *in vivo*, or at pH 7.4 at 37° C is reported to be 2-3 h (ref. 5 and personal communication from Schumacher *et al.*). The maximum aqueous solubility of thalidomide is 20-30 µg/ml.

After feeding with the liquid nutrient medium, tissues were covered with mineral oil ('Klearol'), and cultured at 37° C in 5% CO₂:95% air, in a humidified incubator. Cultures were kept for 3 days, when cartilage was visible in the controls, and then fixed in acetic acid: absolute ethanol (1:3).

The cultures were: (a) mesonephros; (b) mesonephros plus adjacent limb bud; (c) limb buds; (d) metanephric mesenchyme; (e) metanephric bud; (f) metanephric bud recombined with metanephric mesenchyme; (g) embryonic somites, and (h) embryonic somites plus a section of the ventral spinal cord or of the notochord. Although many cells died in older cultures, there was enough viable tissue in 3 day cultures to ascertain tissue differentiation.

Metanephric mesenchyme from 6 week old embryos formed tubules only if cultured in association with the ureteric bud, which corroborates Grobstein's findings⁶ with mouse embryos. Embryonic somites from 6-7 week old embryos formed small amounts of cartilage if cultured alone, but significantly more in association with the embryonic spinal cord or notochord. These results agree with findings using embryonic chick tissues⁷.

Cultures of mesonephros from 6-8 week embryos consistently formed small amounts of cartilage matrix, as shown by staining with Alcian blue, which is diagnostic for glycosaminoglycans. Six experiments were performed with embryos between 6 and 8 weeks old, as judged by size, or the stage of development of tissue fragments found in the debris. The mesonephros was dissected free of adhering tissues, and one side was cut into 1 mm³ pieces and cultured in the presence of thalidomide. The other half was similarly dissected, and cultured either in plain nutrient medium, or with 'Doriden'.

Table 1 Qualitative Scoring for Cartilage Matrix in Cultures of Human Mesonephros

Case No.	Age (weeks)	Treatment	Matrix scoring	
			Range	Cumulative sum
056-5	6+	Thal.	2-3	7
056-7	6+	'Doriden'	3-5	14
056-6	6+	Thal.	2-3	6
056-8	6+	'Doriden'	3-4	11
060-3	7+	Thal.	1-2	4
060-4	7+	'Doriden'	2-3	8
073-1	8+	Thal.	1-3	5
073-2	8+	'Doriden'	1-2	4

The range represents the minimum and maximum values for the three parameters used (amount, density and maturation of matrix). The cumulative sum represents the total for all three parameters. Scoring was from 1 (minimum) to 5 (maximum). The tissues were dissected from 6-8 week old human embryos and cultured on F12X nutrient agar for 3 days. Thalidomide and 'Doriden' were at a concentration of 0.8 mg/100 ml. of medium.

The results are shown in Table 1 and Fig. 1. Cartilage matrix was scored on histological slides, and the following assay procedures were used. The tissue sections stained with Alcian blue were examined independently and "blind" by four scientists and scored on a scale from 1 to 5 with respect to the relative amount of cartilage matrix (that is Alcian blue positive material), the density and the maturation of the matrix. The lowest values represent the least chondrogenic differentiation, and the cumulative sum represents the total of the three parameters (amount, density, maturation) indicating the degree of chondrogenesis. Table 1 shows that chondrogenic inhibition by thalidomide was most noticeable on the younger embryos (6-7 weeks old). The older the mesonephros, the less cartilage formed and the difference between the experi-

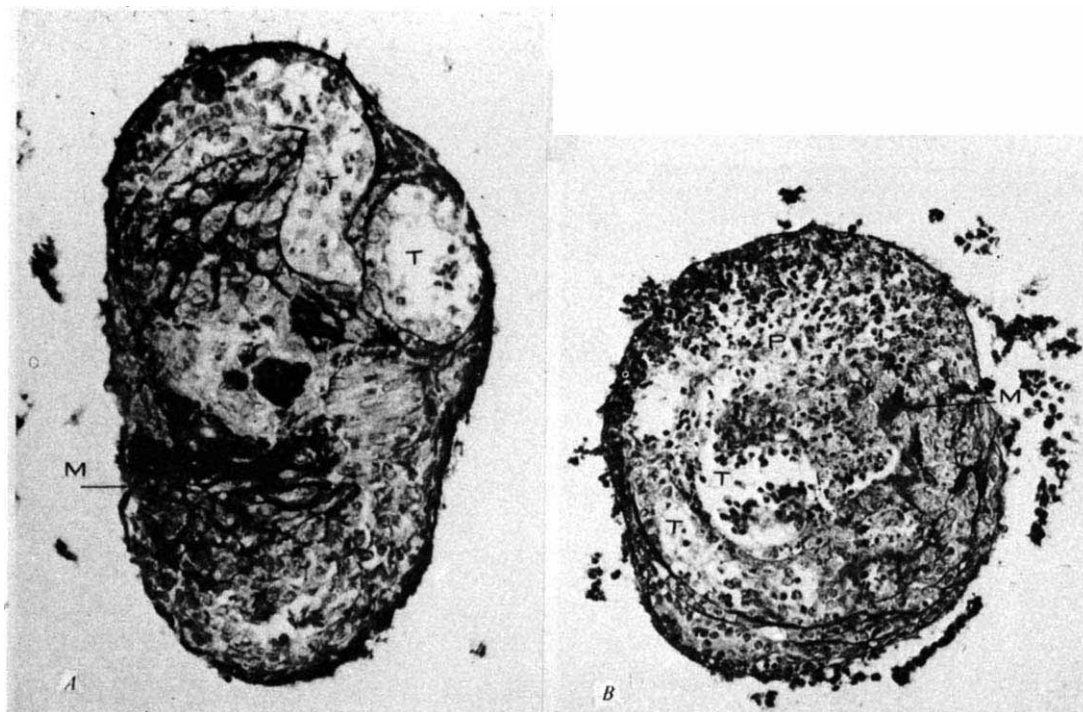


Fig. 1 A, Cultured human mesonephros from a 6 week old human embryo treated with 'Doriden'. The matrix (M) appears black in the photograph and tubules (T) are evident. (Alcian blue $\times 200$.) B, Contralateral mesonephros from the same embryo, treated with thalidomide. The matrix is much less prominent, and the tubules are still evident. Pycnosis (P) is visible. (Alcian blue $\times 100$.)

mentals (thalidomide) and the controls ('Doriden') was negligible.

Thus tissue associated with the human mesonephros at an early stage of development (6–7 weeks of age) seems to form cartilage in tissue culture. As the embryo ages, the amount of cartilage diminishes. The scoring for cartilage matrix in 6 week old tissues cultured in 'Doriden' ranged from 3 to 5 for the three parameters, with a cumulative sum of 14 (Table 1). For 8 week old tissues the corresponding values were 1–2 and 4, indicating significantly less matrix. These results agree well with the results obtained with chick mesonephros³. In addition, the tissues treated with thalidomide showed a significant decrease in matrix formation in 6 week old tissues, a similar decrease in 7 week old tissues, but no difference in 8 week old tissues. This again is similar to chick mesonephros³.

When human limb buds from these embryos were grown in culture, cell death was more rampant, but nevertheless cartilage formed and there was no noticeable effect of thalidomide. None of the other chondrogenic tissues were inhibited.

These results suggest that a major site of action of thalidomide on limb-related chondrogenesis in human embryos is tissue associated with the embryonic mesonephros at a certain stage of development.

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JAMES W. LASH
LAURI SAXÉN

Department of Anatomy,
School of Medicine,
University of Pennsylvania,
Philadelphia, Pennsylvania 19104, and
III Department of Pathology,
University of Helsinki,
Helsinki SF00290

Received March 16; revised May 12, 1971.

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Rabbit Blastocoele Perfusion Technique

TROPHOBLAST cells must play a significant role in the movement of solute and water into the blastocoele¹. The epithelial trophoblast (Fig. 1A), exposed on one side to the uterine environment and on the other to the blastocoele fluid, appears structurally and functionally similar to other solute and water transporting organs with epithelial cells which separate the blood stream from body cavities². There is evidence for active ionic transport across the rabbit trophoblast^{2–5}, perhaps in connexion with the accumulation of blastocoele fluid^{2,4}.

The active transport components of the trophoblast membrane could be studied by techniques which have been applied successfully to various epithelial membranes, if the composition of the blastocoele fluid could be controlled. We have therefore investigated this problem. My first approach was to form a flat sheet of cells out of the blastocyst which could be placed between two fluid chambers, similar to the method used to study frog skin epithelium. My second method was to perfuse the blastocoele cavity using glass pipettes. Perfusion methods have been developed for various tissues, such as the squid giant axon⁶, marine algae⁷, and isolated renal tubules⁸. After many attempts at both approaches, the latter proved to be the better solution. I now describe a successful method and report electrical parameters of the 6 day rabbit blastocyst under continuous-flow perfusion.

Blastocysts were flushed from the uteri of naturally mated New Zealand rabbits. One of them (2–3 mm, average diameter) was secured in a special environmental chamber⁴ and glass perfusion pipettes (40–60 μ m tip diameter) were inserted into

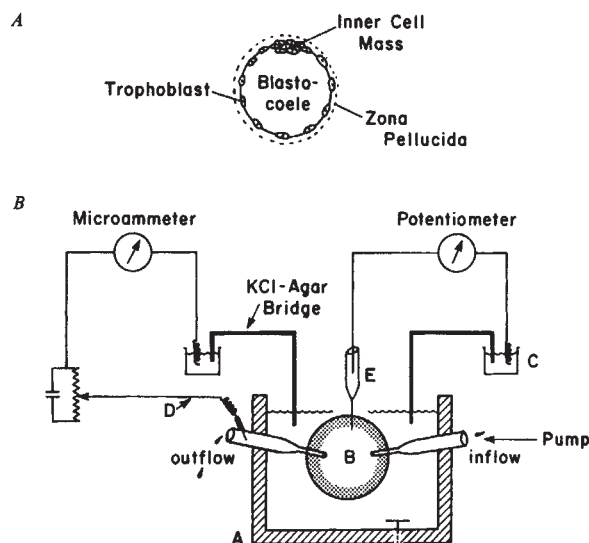


Fig. 1 A, Cross section of a 6 day blastocyst. B, Assembly for internal perfusion and electrical measurements of the blastocyst. A, Chamber used to secure embryo and maintain constant temperature, humidity, and gas phase (5% CO₂ in air); B, blastocyst; C, Ag-AgCl-KCl half cell; D, Ag-AgCl wire used for applying outside EMF; E, microelectrode.

opposite sides of the blastocyst. All manipulations were carried out using micromanipulators under a dissecting microscope.

The two chief difficulties in the perfusion of the blastocoele are: (1) how to penetrate the zona pellucida and trophoblast with pipettes which are both large enough in tip diameter to allow relatively low resistance to perfusion flow, and small enough not to cause the embryo to collapse when penetrated; (2) how to prevent leaks at the junction between the trophoblast and the pipette.

The solution was first to penetrate the tough inert zona pellucida and the trophoblast layer with a micropipette (tip diameter < 1 μ m), and then advance the micropipette into the blastocoele cavity until the shank of the micropipette was of similar diameter to that of the inflow pipette to be used. The micropipette was then withdrawn, leaving a small wound in the trophoblast wall and a small opening in the zona. The inflow pipette, filled with medium, connected to the inflow reservoir and clamped, was then inserted through the opening in the zona and the wound in the trophoblast without collapse of the blastocyst and with very little loss of blastocoele fluid. Using the same procedure, the outflow pipette, filled and connected to the outflow reservoir, was inserted on the opposite side. Any fluid loss from the blastocoele could now be replaced by opening the input side of the perfusion system and pumping in fluid while maintaining the output side closed. Once the perfusion pipettes were in place, a microelectrode was inserted into the blastocoele cavity for electrical measurements. The trophoblast epithelium was then allowed to seal around the three inserted pipettes (Fig. 2).

Surprisingly, the trophoblast epithelium adhered naturally to the glass pipettes forming a tight seal. By regulating the inflow perfusion rate by adjusting the pumping speed and the outflow rate by regulating the height of the outflow reservoir in relation to the chamber, the blastocyst could be maintained at its original volume without leakage around the trophoblast-pipette junction. By regulating inflow and outflow rates in this manner, the blastocoele contents could all be removed so that the cavity collapsed, and then replaced almost at will. Leaks around the pipettes could be detected microscopically and electrically. If perfusion pressure were increased, the blastocoele cavity expanded, causing the junction between the trophoblast and the pipette to be broken and the potential to decrease rapidly to near zero. Finally, to assess the possible leakage of the perfused blastocyst, ¹⁴C-sucrose was added to the

inflow reservoir and the outside solution was tested for the appearance of radioactivity. In sixteen collections in four preparations no activity was detected in the bathing solution, showing that a sensitive perfusion control system eliminated leakage problems. The perfusion procedure was started by quickly replacing the contents of the blastocoele with perfusion solution, after which a constant perfusion rate was maintained. Flow rates averaged between 2 and 3 μ l./min. In a blastocyst of 2.5 mm average diameter there would be a complete exchange of solution about every 3–4 min.

Table 1 Electrical Properties of the Perfused Rabbit Blastocyst

Blastocyst	Time (min)	E_t * (mV)	I_{sc} (μ A/cm ²)	R (Ω cm ²)
1	210	-6.2	5.2	1,204
2	70	-4.4	2.9	1,509
3	90	-4.8	2.8	1,783
4	150	-4.4	3.8	1,237
5	70	-5.7	3.0	2,218
6	110	-4.3	3.6	1,824
7	90	-6.6	6.5	1,099
8	180	-8.0		
9	180	-9.7		
10	180	-5.7		
11	90	-8.2		
12	130	-4.1		
Mean $\pm$ s.e.		-6.0 $\pm$ 0.52	4.0 $\pm$ 0.52	1,553 $\pm$ 154

* Blastocoele cavity negative with respect to external medium.

E_t and I_{sc} were recorded at 10 min intervals throughout each experiment.

The potential difference (E_t) across the trophoblast epithelium, short-circuit current (I_{sc}) and resistance (R) are given along with the time during which E_t and I_{sc} remained relatively constant. The perfusion and bathing medium were Eagle's containing 20% calf serum.

When experience with the technique permitted a high frequency of successful perfusion, the electrical properties of the epithelial trophoblast were studied. The potential difference (E_t) and short-circuit current (I_{sc}) across the trophoblast wall were measured as described by Ussing and Zerahn⁹ with the circuit shown in Fig. 1B. Eagle's medium containing 20% calf serum was used for the perfusion and outside bathing media. The trophoblast membrane behaved as a simple linear resistor, and so resistance measurements were computed from the ratio of open-circuit potential to short-circuit current (Fig. 3). The resistance of the bathing solution measured without the blastocyst in position was always less than 40 Ω cm.

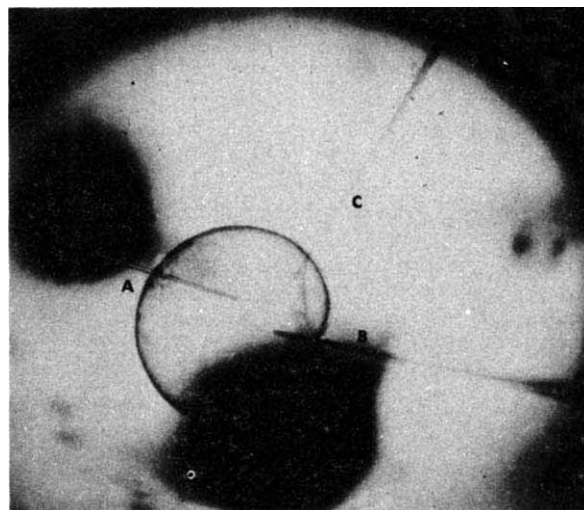


Fig. 2 Perfusion of a 6 day rabbit blastocyst during measurement of the electrical properties of the trophoblast wall. A, Inflow perfusion pipette; B, outflow pipette; C, microelectrode. The two dark spots in the photograph are caused by two gaskets at the bottom of environmental chamber.

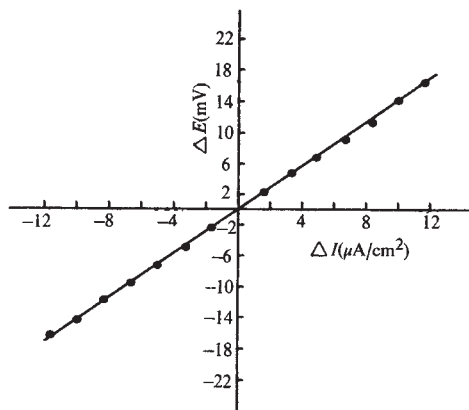


Fig. 3 A typical current-voltage graph for a perfused rabbit blastocyst. The origin of potential is -4.4 mV. Negative values of ΔE represent hyperpolarized potentials, produced by current passing inward across the trophoblast.

Table 1 shows the electrical properties of continuously perfused rabbit blastocysts. The average potential across the epithelial trophoblast ($\pm$ s.e. of the mean), of twelve blastocysts under continuous perfusion with identical solutions on both sides, was -6 ± 0.52 mV, whereas the value for twenty-three non-perfused embryos was -11.1 ± 0.79 mV. The average specific membrane resistance of seven blastocysts measured under continuous perfusion was $1,553 \pm 154 \Omega\text{cm}^2$ and compares with $2,188 \pm 202 \Omega\text{cm}^2$ averaged from fifteen non-perfused embryos. In many cases, a non-perfused and a perfused blastocyst were obtained from the same rabbit. The average short-circuit current recorded in seven perfused blastocysts was $4 \pm 0.52 \mu\text{A}/\text{cm}^2$ or about $0.15 \mu\text{equiv.}/\text{cm}^2/\text{h}$. When both sides of an epithelial membrane are bathed with identical solutions, the short-circuit current represents the net active movement of ions across that membrane⁹. The time during which both E_t and I_{sc} remained relatively constant averaged 129 min lasting 70–90 min in five embryos and 110–210 min in seven others (Table 1). Some embryos may have maintained steady electrical properties longer than the times shown (180–210 min) for these experiments were arbitrarily terminated. Three blastocysts, which had their zonae pellucidae mechanically removed before perfusion, showed electrical properties similar to those with intact zonae. A technique has therefore been developed which will permit the study of transport phenomena across a mammalian embryonic membrane, using methods which are well documented for other epithelial membranes.

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MORTON H. CROSS

Laboratory of Reproductive Physiology,
School of Veterinary Medicine,
University of Pennsylvania,
Philadelphia,
Pennsylvania 19104

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Limited Synergism between Rat Thymus and Mouse Bone Marrow

In a number of experimental systems¹ it has been shown that thymus and bone marrow-derived cells can cooperate in the production of humoral antibodies. In some instances this synergism has been demonstrated when the interacting cells are antigenically similar but in other experiments allogeneic cells have been found to cooperate. We have now demonstrated cooperation between rat and mouse cells. A two-step experiment was performed, following the design of Miller². Rat or mouse thymus cells were introduced with antigen into irradiated recipients. The donor cells were collected and injected into secondary host mice. The details of these experiments and their results are as follows.

Twenty adult RK mice, 10–12 weeks old, were given 900 r. of total body irradiation from a ⁶⁰Co source. Ten were injected with 2×10^7 mouse thymus cells plus 0.1 ml. of SRBC at 20% (group 1), and ten were injected with 2×10^7 rat thymus cells plus 0.1 ml. of SRBC at 20% (group 2).

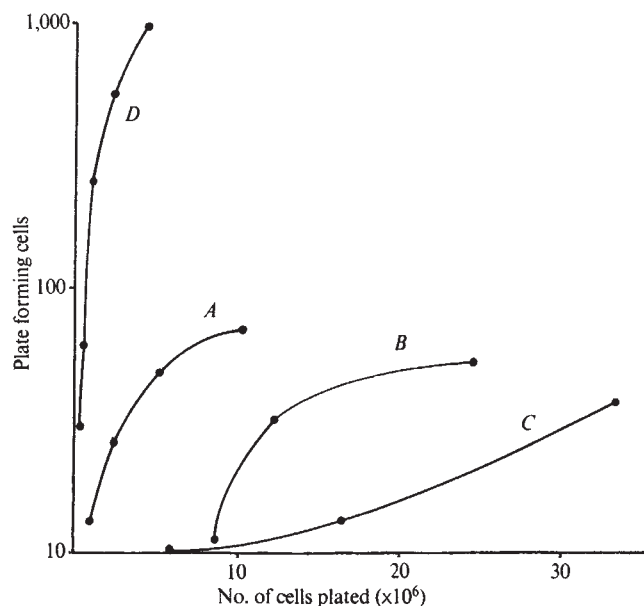


Fig. 1 Effect of mouse and rat thymus on the immunocompetence of mouse bone marrow cells (see text). Group A lethally irradiated mice repopulated with mouse thymus and mouse bone marrow cells; group B, lethally irradiated mice repopulated with rat thymus and mouse bone marrow cells; group C, lethally irradiated mice repopulated with mouse bone marrow cells; group D, unirradiated control mice given only SRBC.

Five days later another twelve RK mice, 10–12 weeks old, were given total body irradiation, and then three were injected with 1.5×10^6 cells from group 1 pooled spleens plus 2×10^7 mice bone marrow cells and 0.1 ml. of SRBC at 20% (group A), three were injected with 1.5×10^6 cells from group 2 pooled spleens plus 2×10^7 mice bone marrow cells and 0.1 ml. of SRBC at 20% (group B), and three were injected with 2×10^7 mice bone marrow cells and 0.1 ml. of SRBC at 20% (group C). As controls three irradiated and three non-irradiated mice (group D) were immunized with 0.1 ml. of SRBC at 20%.

Six days later we counted plaque forming cells against SRBC—of pooled spleens of the different groups—by the Jerne technique³. All cellular suspensions were prepared in cold phosphate buffer saline, P 7.4, and injected intravenously within 3 h of the irradiation.

Fig. 1 shows that rat and mouse cells can cooperate, although not as efficiently as mouse-mouse combinations. The mechanism of cooperation is not understood but if it involves recog-

nition of organ specific surface antigens it seems that these may be recognized across genetic barriers.

FERNANDO MORGADO
HUGO FOLCH

Department of Experimental Medicine,
Austral University of Chile,
Box 867, Valdivia

Received March 25; revised May 5, 1971.

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Cytotoxic Antibody for Epithelial Cells in Human Graft versus Host Disease

THE transplantation of immunocompetent cells into an immunologically suppressed individual frequently results in a syndrome believed to be due to an immune reaction of the grafted cells against the host. In man this graft versus host (GVH) reaction prominently involves the skin, gastro-intestinal tract and liver, and is characterized pathologically by lymphoid cell infiltrates, basal cell layer destruction and dyskeratosis¹. The mechanism by which the GVH reaction damages epithelial structures has not been studied in man. Streilein and Billingham have studied a GVH-like reaction prominently involving the skin in hamsters and have made a number of observations pertinent to the mechanism of the GVH pathology^{2,3}. One of their observations, the finding of a cytotoxic factor against hamster and rat epidermal cells in the serum of hamsters with GVH-like skin lesions stimulated us to investigate this in man. We have found in human patients with GVH an IgM antibody which is cytotoxic for epithelial but not lymphoid cells.

We studied sera from recipients of bone marrow transplants being cared for on the Leukemia Service of the National Cancer Institute. Of eight patients who underwent bone marrow transplants from their HL-A identical siblings, four developed clinical manifestations compatible with GVH, and in three of these the diagnosis was confirmed pathologically⁴⁻⁶. Sera were tested for cytotoxicity using a ⁵¹Cr cytotoxicity technique⁷. Target cells labelled with ⁵¹Cr and serum were incubated for 10 min before addition of rabbit complement, and then for 90 min at room temperature. A given serum was considered to be cytotoxic if it caused a minimum of 15% more ⁵¹Cr release from the target cells than normal human serum. The target cells used were monolayer cultures of HeLa and Hep-2 cells, both epithelial cell lines derived from a squamous cell carcinoma of the cervix and a squamous cell carcinoma of the larynx respectively. Lymphoid target cells were from lymphoid cell lines maintained in long term culture.

Reactivity of one patient's serum with epithelial and lymphoid cells at various intervals after transplantation is shown in Fig. 1. Pretransplant serum contained no cytotoxicity against either lymphoid or epithelial cell lines. During the third week after grafting, however, his serum contained cytotoxicity against the epithelial cells. No cytotoxic reactivity against lymphoid cells was present. The appearance of epithelial cytotoxicity coincided with the development of a rash, diarrhoea, jaundice and desquamation, all of which seemed compatible with GVH reaction. A skin biopsy on the twentieth day after the transplant as well as autopsy findings in skin, liver and intestine on the 32nd day were all compatible with GVH disease.

Two further patients had clinical evidence of GVH which was confirmed pathologically, and a third had mild symptoms

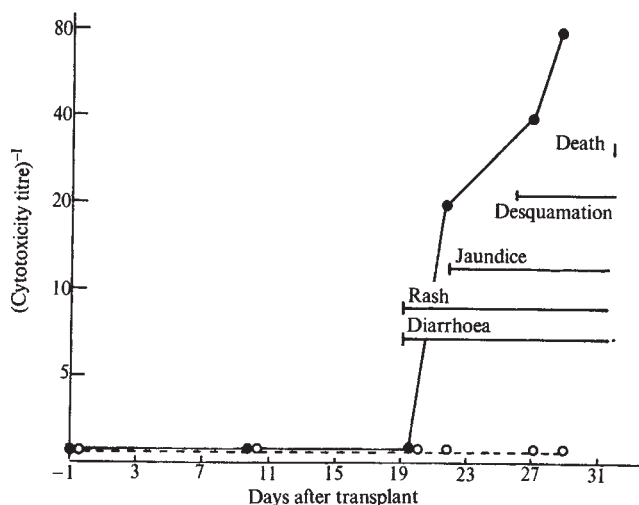


Fig. 1 Cytotoxic activity of serum from a patient with GVH following bone marrow transplantation. The solid line shows reactivity against epithelial (HeLa and Hep-2) cells; the broken line reactivity against lymphoid cells. The onset of each manifestation of GVH is also shown.

compatible with GVH, although the diagnosis was not confirmed pathologically. In each of these patients, serum cytotoxicity against epithelial but not lymphoid cells appeared coincident with the development of rash and other manifestations of GVH. In contrast with sera from patients with GVH, sera from four transplant recipients in whom there was graft take but no GVH were not cytotoxic for epithelial cells. Similarly, sera from fifteen patients with acute lymphocytic leukaemia who did not receive marrow transplants, and sera from fifteen normal individuals were not cytotoxic for epithelial cells (Table 1).

Table 1 Antiepithelial Activity of Sera from Patients with GVH

Epithelial cell antibody (No. positive/No. tested)	Patient group studied
3/3	Clinical GVH, confirmed histologically
1/1	Probable GVH, not confirmed histologically
0/4	Graft take without evidence of GVH
0/15	ALL not grafted
0/15	Normal individuals

Sera were from non-affected transplant recipients, non-transplanted leukaemics, and normal individuals. ALL, acute lymphocytic leukaemia.

The cytotoxic factor was characterized with respect to molecular properties and specificity. The cytotoxicity of the sera for epithelial cells was complement dependent. Reactive sera from two of the patients were fractionated by gel filtration through 'Sephadex G-200'. The cytotoxic activity was found in the excluded protein peak which contained IgM but not IgG or IgA when screened by Ouchterlony agar diffusion using specific antisera. Treatment of cytotoxic sera with 0.1 M 2-mercaptoethanol destroyed the ability of the sera to lyse epithelial cells. These results provide evidence that the cytotoxic factor in these patients with GVH disease is an IgM antibody.

To investigate the possibility that the sera were reacting against an HL-A antigen, they were screened against a panel of lymphocytes from ninety individuals; no pattern of HL-A reactivity was observed. In addition, absorption of the sera with lymphocytes having a broad range of HL-A alloantigens did not remove the cytotoxicity for the epithelial cells. Absorption of the reactive sera with one epithelial cell line removed reactivity for the other. Thus it seems that the GVH antibody

is not directed against HL-A antigens but has specificity for epithelial cells. A further indication of this specificity is the finding that the sera failed to react with tissue culture hepatocytes and fibroblasts.

The pathogenesis of the GVH reaction is unknown; but the observation that patients with this disease have an antibody directed to epithelial cells suggests several mechanisms for this reaction. The antibody we are detecting may be directed against minor histocompatibility antigens that are expressed only as epidermal specific antigens not detected by the usual HL-A matching techniques. Boyse *et al.* have presented data suggesting similar antigens in mice⁸. Alternately, it may be that damage initially produced in the skin by the GVH reaction exposes hidden epidermal antigens and these then provoke the formation of "autoantibodies" against epithelial cells. A third possibility is that impaired immune function in the grafted recipient predisposes to certain viral infections, and that alteration of epidermal cell surface antigens induced by virus, or cross-reactivity between viral and epidermal antigens accounts for the antibody detected in these patients. Further investigation of these and other possibilities may provide information about the importance of tissue specific non-HL-A antigens and antibodies in the processes of graft rejection and in the pathophysiology of the GVH reaction.

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C. B. MERRITT
D. L. MANN
G. N. ROSENTINE, JUN.

Immunology Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda,
Maryland 20014

Received December 31, 1970; revised March 15, 1971.

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Teratocytes as a Means of Resistance to Cellular Defence Reactions

I HAVE suggested that teratocytes, the giant cells which develop from the trophamnion of many parasitoid Hymenoptera, serve as a means of resistance to the defence reactions of insect hosts¹. These cells are relatively small when they dissociate from the embryonic membrane, but as they become distributed in the blood of the host they expand to as much as 3,000 times their original volume. Their growth is achieved by the absorption of nutrients from the host's blood and I suggested that the consequent depletion of materials in the haemolymph would impede or inhibit haematopoiesis and prevent an effective haemocytic reaction to the young parasitoid.

Evidence supporting the hypothesis has been obtained in the course of experiments designed for another purpose. Eggs and larvae of the ichneumon wasp *Nemeritis canescens*, which does not produce teratocytes, were implanted in caterpillars of *Eucosma hohenwartiana* collected out of doors from heads of knapweed, *Centaurea nigra*. In healthy caterpillars the parasitoid was promptly killed by cellular defence reactions. Against all expectation, one larva of *Nemeritis* was found feeding and developing without encapsulation when it was 5 days old. The caterpillar containing this individual, however, was also infected by a braconid parasitoid, and its blood contained numerous teratocytes about 50 μ m in diameter. In two caterpillars infected by wild parasitoids which had not produced teratocytes, the *Nemeritis* was encapsulated as it was in healthy hosts.

Three more examples of this phenomenon have recently been observed in caterpillars of *Chilo phragmitellus* collected from reeds at Wicken Fen. When eggs of *Nemeritis* were implanted in healthy caterpillars of this species, they were encapsulated, melanized and killed; only two hatched, and the larvae were then promptly destroyed in the same way. Three caterpillars in which eggs were implanted turned out to be already parasitized by gregarious braconids, probably *Apanteles ferrugineus*, which had produced many teratocytes. From these three hosts, and from them only, larvae of *Nemeritis* 5 days old were retrieved alive, feeding and apparently developing normally, without any trace of a haemocytic defence reaction.

These observations show that teratocytes are indeed a means of resistance to the cellular defence reactions of insects, and that the protection they afford is not restricted to the species of parasitoid that produces them but extends to other species. Since their action is not specific, attrition of the host is most likely to be the mechanism by which teratocytes have their protective effect.

I thank Mr J. A. Lade for help with the collection of caterpillars.

GEORGE SALT

Department of Zoology,
University of Cambridge

Received April 13, 1971.

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Cellular Basis for Immunologic Memory

AFTER exposure to an antigen resulting in a primary immune response, experimental animals and human subjects possess the ability to react specifically and in accelerated fashion to a second exposure to the same antigen. This immunological memory presumably involves specific cells, but it is not known whether it involves generation of an increased number of cells or increased synthetic (or some other) ability in individual cells. Nobody has yet identified a cell which was generated by a previous contact with a specific antigen. Lymphocytes present in the circulation of rats have been shown to carry memory¹, and recent work has established that many such cells are thymus-derived and important in many aspects of the immune response^{2,3}. Because they are long lived and constantly re-circulating throughout the peripheral lymphoid organs, thymus-derived cells make ideal candidates for the carriage of memory. Indeed, memory can be diminished by killing cells in this sub-population⁴.

Exposure to an antigen results in a burst of mitosis in the lymphoid organs, and at least initially many of the cells in mitosis are thymus-derived⁵. Moreover, these cells respond

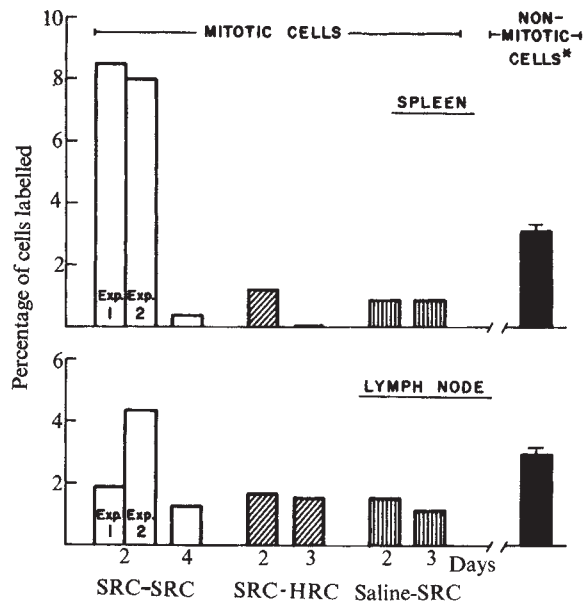


Fig. 1 Percentages of labelled mitotic and non-mitotic cells in spleen and mesenteric lymph nodes following secondary challenge. Results of two experiments are shown. The results are pooled from all the groups for they were all quite similar. The bar indicates the standard error obtained from meaning the seven groups.

with an accelerated burst of mitoses in the secondary response³. The peak of the mitotic response to sheep red cells (SRC) occurs on day 3 in the primary response and on day 2 in the secondary. The role of the mitosis is unknown, but it may involve the generation of specific cells some or all of which are destined to carry immunological memory. We have investigated this possibility by isotopically labelling cells synthesizing DNA in a primary response and observing the numbers of such labelled cells which responded mitotically to a second antigenic challenge. In doing this we took advantage of the work of Davies *et al.*^{3,5}, which clearly delineates the periods of peak mitotic activity of thymus-derived cells in mice following primary and secondary antigenic challenge. Thus we administered label for 4 days immediately after sensitization with SRC and re-stimulated with this antigen 3 weeks later. At this time we could expect continually dividing cell lines to have lost their label by dilution, while non-dividing cells would not. After a course of labelling with ³H-thymidine similar to that described here, a population of heavily labelled lymphocytes is found in the spleen and lymph nodes of rats which diminishes in numbers very slowly with time⁶⁻⁹. By 21 days after cessation of labelling, when the initial rapid loss of label subsides, only a small population of heavily labelled cells persists⁶⁻⁹.

Adult CBA mice received either 5×10^8 SRC or saline intraperitoneally, followed by injections of ³H-thymidine every 6 h for 4 days (total dose = 7 μ Ci of 17.8 Ci/mmol specific activity of ³H-thymidine/g body weight). Two groups of four mice, one immunized and one control, were given colcemid 3 h after the last thymidine injection. These animals were

killed 1.5 h later and their spleens and mesenteric lymph nodes were removed. These tissues were divided roughly equally—half was used for tissue sectioning and the other processed by Ford's method¹⁰ to give mitotic spreads. All other mice were challenged intraperitoneally with either 5×10^8 SRC or the same number of horse red cells (HRC) 3 weeks after primary immunization. On the second, third and fourth days after challenge, groups of three or four mice were killed and their spleens and lymph nodes processed in the same manner. All sections and spreads were dipped in diluted Kodak NTB3 nuclear track emulsion, exposed for 3.5 weeks, and developed in Kodak D19. Sections were stained with 0.1% toluidine blue and spreads with 2.0% aceto-orcin. The spreads were scored in a "blind" fashion by four persons, at least 1,500 non-mitotic nuclei and 300 mitoses being counted in each experimental group. Grain counts were performed in individual nuclei.

In an additional control experiment, two groups of six mice were immunized with 5×10^8 SRC intraperitoneally and one of these groups was given ³H-thymidine as described above. All animals were bled weekly and then challenged after 3 weeks with 5×10^8 SRC and bled 5 days later. The immune response, assessed by haemagglutination titrations, was not altered by the injections of isotope.

Table 1 shows the distribution of label in the spleen cells of immunized and non-immunized mice killed immediately after the cessation of labelling (day 4). It can be seen that animals given SRC contain nearly twice as many labelled cells as control animals. In tissue sections, 4 days after the primary antigenic stimulus, many more labelled large lymphoid cells were present in the paracortical zones of mesenteric lymph nodes and the splenic white pulp than in animals given label but not antigen. There was also a diffuse increase in labelled reticulum cells in the marginal zone and red pulp of the spleen.

After specific challenge, that is, a secondary injection of SRC, very much greater numbers of labelled cells were found in mitosis in the spleens of animals at the peak of the mitotic response of thymus-derived cells³ than were found in the spleens of animals originally given SRC and now challenged with HRC, or animals undergoing a primary immune response to SRC (Fig. 1). For the purpose of this communication a labelled nucleus is one which contained more than ten grains, although the average number of grains/labelled cell was less than seen immediately after the cessation of labelling. The numbers of labelled non-dividing cells in each of these groups did not differ significantly. Only in the group undergoing a secondary response to SRC was the proportion of labelled cells in mitosis greater than the proportion of labelled non-dividing cells. In spreads prepared from the mesenteric lymph nodes, a specific response of lower degree was observed in only one of the two experiments (Fig. 1). In histological sections of the spleen, labelled mitoses were limited to the white pulp. None was found in the germinal centres, and tingible bodies were also unlabelled. The labelled, non-dividing cells of the marginal zone and red pulp were equally numerous in specific animals and controls. In the lymph nodes, labelled mitotic cells were limited to the paracortical lymphoid tissue. By 4 days, when the mitotic activity of thymus-derived cells is over³, labelled mitoses could no longer be found in either spreads or sections.

These results indicate that cells from the long lived popula-

Table 1 Labelling of Spleen Cells 4 Days after Induction of a Primary Response to SRC

Immunization	0-5*	Non-mitotic cells (%)				Mitotic cells (%)				
		6-10	11-20	21-40	> 40	0-5	6-10	11-20	21-40	> 40
None	84.0	1.0	2.7	4.4	7.9	0	0	0	0	100
SRC	75.3	1.5	1.9	5.4	15.9	0	0	0	4.5	95.5

* Number of grains/cell.

tions, which are stimulated to synthesize DNA by contact with antigen, enter a resting phase until stimulated to re-enter division by secondary exposure to the same antigen. By definition these would be memory cells. They apparently correspond to Mitchell and Miller's "educated T lymphocytes"², for they were only observed at a time when the mitotic response of thymus-derived lymphocytes is maximal³. We are currently seeking direct experimental proof of their identity. Previous attempts to establish this point¹¹ have failed, perhaps because of the use of *Salmonella* antigens, which are poor stimulators of thymus-derived cells¹². Our finding implies that cell replication may serve as a means of enlarging and/or altering the pool of thymus-derived cells capable of reacting with certain antigens. Replication may not, however, be essential to the helper function of these cells¹³.

At the peak of the secondary response to SRC 30–40% of the cells in mitosis in the spleen are thymus-derived³. Thus we were initially surprised to find only 8–9% of the spleen cells responding mitotically to a second injection of antigen which showed evidence of having responded in the earlier primary response. It is important to remember that the work delineating the time and magnitude of the response of thymus-derived cells to antigen was carried out in irradiated, thymus-grafted mice. Thus it is possible that there may be some differences in normal mice. If there should be any significant differences, however, there would probably be an increase of thymus-derived cells responding in normal mice. Davies *et al.* have shown that increasing the number of reconstituting thymus grafts up to a point increases the number of peripheral thymus-derived cells³. This increase does not, however, alter the percentage of thymus-derived cells responding to antigen in lymph nodes nor does it change the timing of their response. It is possible that some thymus-derived memory cells lost their label through division after the period of labelling. We are investigating the possibility of recruitment of thymus-derived cells by other thymus-derived cells responding specifically. The small number (0–1%) of labelled cells found in control animals may be due, at least in part, to the emergence and subsequent response of a few thymus-derived cells which were undergoing division in the thymus at the time of thymidine administration.

The chief conclusion we should like to draw from this experiment is that one result of the DNA synthesis which follows primary antigenic stimulation is the generation of memory cells. The ability to identify these cells should be of value in characterizing them further.

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Note added in proof: Since we submitted this manuscript, another report in which memory cells were identified by autoradiography, has appeared. Bosmann, C., and Feldman, J. D., *Clin. Exp. Immunol.*, **7**, 565; 1970.

R. K. GERSHON
J. KRÜGER
J. D. NAYSMITH
B. H. WAKSMAN

Departments of Pathology and Microbiology,
Yale University School of Medicine,
New Haven,
Connecticut 06510

Received November 23, 1970; revised March 10, 1971.

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Polychlorinated Biphenyls: Photolysis of 2,4,6,2',4',6'- Hexachlorobiphenyl

MANY aromatic organochlorine compounds are readily utilized or otherwise decomposed by a number of specific microorganisms. In the environment, however, these compounds are relatively stable and resistant to general microbial breakdown. For many of these compounds photochemical degradation, induced by the ultraviolet content of the Sun's light, may be a major pathway of decomposition.

Because a large number of pesticides contain a chloroaromatic moiety as a primary structural feature, considerable attention has been paid to the photolysis of these compounds^{1–7}. Photochemical decomposition of chlorobiphenyls may be a significant process in the environment because they absorb in that region of the ultraviolet (ref. 8 and our unpublished demonstrations) which is available in natural conditions (compare ref. 1) from sunlight.

Commercially available polychlorinated biphenyls (PCB) for example 'Aroclor', consist of a complex mixture of chlorinated biphenyls which can be resolved into more than fifty peaks by gas chromatography on 'Scot columns' (private communication from D. J. Sissons and G. M. Telling). As it is difficult to correlate data from such a complex mixture we have synthesized a series of isomeric di, tri, tetra, penta, hexa, octa and decachlorobiphenyls to facilitate fundamental studies on analytical, biological and chemical aspects of PCB (manuscript in preparation). We have also shown that all these compounds give strong molecular ions on electron

Table 1 GLC and Mass Spectral Data for Hexachlorobiphenyl Photolysis Products

	Retention time* (min)	m/e of M ⁺	Chlorine† content
TLC band I			
Peak 1	5.6	—‡	0
Peak 2	8.2	—‡	0
Peak 3	9.9	222	2
Peak 4	13.5	256	3
Peak 5	14.1	290 (256)	4, (3 ^{tr})
Peak 6	19.0	324 (290)	5, (4 ^{tr})
Peak 7	29.7	358	6
Peak 8	56.9	304	2
TLC band II			
Peak 1	19.6	290	4
Peak 2	26.8	324	5
Peak 3	35.2§	358	6

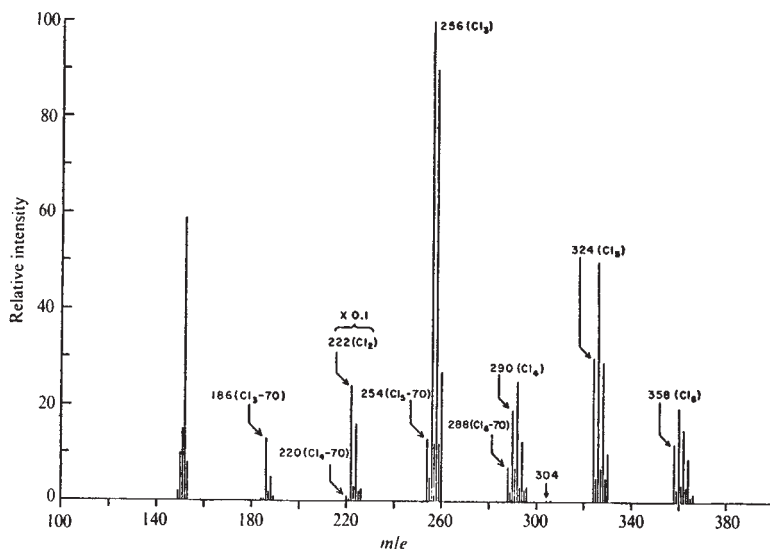
* Conditions: instrument, Hewlett-Packard 5750; column, 6% QF-1+4% SE30 on 'Chromosorb' (AW) 60–80 mesh 5', 1/4" outside diameter, glass; injection port temperature, 220° C; column temperature, 175° C; flame ionization detector temperature and collection port temperature, 240° C; helium flow rate, 40 ml./min.

† From isotope peak distribution; tr, trace.

‡ Hydrocarbon peaks, M⁺ not identified.

§ Corresponding to starting material.

Fig. 1 70 eV mass spectrum (DuPont/CEC 21-110B instrument). The temperature of the sample, controlled independently from the source temperature¹⁰, was 40° C. The intensity of ions corresponding to the components ($\text{Cl}_6 = \text{C}_{12}\text{H}_4\text{Cl}_6$, $\text{Cl}_5 = \text{C}_{12}\text{H}_5\text{Cl}_5$, and so on) in the mixture varies with sample temperature. Each isomer shows an $\text{M}^+ - 70$ ion as the most important fragment. These ions are indicated as $\text{Cl}_6 - 70$, $\text{Cl}_5 - 70$, and so on.



impact, followed by less intense ions resulting from successive loss of Cl and/or HCl (manuscript in preparation). As the general formula for PCB is $\text{C}_{12}\text{H}_{10-x}\text{Cl}_x$ the molecular ions of individual isomers are thus readily recognized in a mass spectrum of a mixture of these compounds (compare ref. 9).

We now wish to report the photochemical behaviour of 2,4,6,2',4',6'-hexachlorobiphenyl. A sample of 25 mg of this compound in 2.0 ml. of hexane was irradiated at λ_{max} 3100 Å for 100 min. Thin-layer chromatography, using 'Merck Silica Gel F-254' and hexane, revealed the presence of two major bands; band I (R_F 0.40) and band II (R_F 0.65, corresponding to starting material). The mass spectrum of band I (Fig. 1) indicated molecular ions corresponding to di, tri, tetra, penta and hexachlorobiphenyls. Gas liquid chromatography (GLC) of bands I and II revealed eight and three major peaks respectively. Retention times and mass spectra data for all peak components are reported in Table 1. When irradiation was carried out in methanol, similar results were obtained, but in addition a third and more polar band appeared, containing oxygenated polychlorinated compounds with structures which have not been investigated further.

Two conclusions can be drawn from the results of this study: (i) during photolysis, chlorine is removed rather readily from hexachlorobiphenyl yielding compounds of lower chlorine content; (ii) photochemical production of new compounds formed by loss of chlorine, rearrangement or condensation may lead to further complications in residue analysis with the possible appearance of chlorobiphenyls ("peaks") in environmental samples that are not present in commercial PCB mixtures.

S. SAFE
O. HUTZINGER

Atlantic Regional Laboratory,
National Research Council of Canada,
Halifax, Nova Scotia

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Selection for Resistance to Carbamate and Organophosphorus Insecticides in *Anopheles albimanus*

THE development of resistance in anopheline mosquitoes to DDT, HCH, and cyclodienes, and the consequent set back in malaria eradication programmes has lent urgency to the search for alternative insecticides to replace the chlorinated hydrocarbons. During the past few years, more than 1,300 new insecticides, chiefly organophosphates and carbamates, have been tested under an international collaborative programme of the World Health Organization¹. Testing consists of seven sequential stages and only insecticides that satisfy the criteria in all stages are recommended for use on a large scale.

One of the insecticides that satisfied the criteria of stage VI (operational evaluation) and has been found suitable for stage VII (epidemiological evaluation) is the carbamate, propoxur². It is intrinsically more toxic to adults of many mosquito species than dieldrin, malathion, fenitrothion, and as a residual spray has been found to be effective for 2 to 4 months against *Anopheles gambiae* and *A. funestus* in Africa, *A. stephensi* and *A. dhali* in Iran, and *A. albimanus* in El Salvador².

No case of resistance to carbamate or organophosphorus insecticides has yet been reported although tolerance to malathion has been suspected in *A. albimanus* in Guatemala and Nicaragua^{3,4} and subsequently demonstrated in El Salvador¹⁶. In this communication we report the development of high degrees of carbamate and organophosphorus resistance in *A. albimanus* resulting from intensive selection pressure in the field, and subsequently in the laboratory.

Earlier attempts to induce carbamate resistance in *A. albimanus* by laboratory selection have been unsuccessful, although in *Culex pipiens fatigans* a 30× level of resistance to propoxur developed in larvae within twenty-five generations of selection pressure^{5,6}. A strain of *A. albimanus* from Panama selected with *m*-isopropylphenyl methylcarbamate for fifty generations developed only a 2.8× increase in the LC_{50} level⁷. Another strain from Haiti, containing dieldrin-resistant individuals, when subjected to larval selection pressure by propoxur for nineteen generations and to adult pressure during the

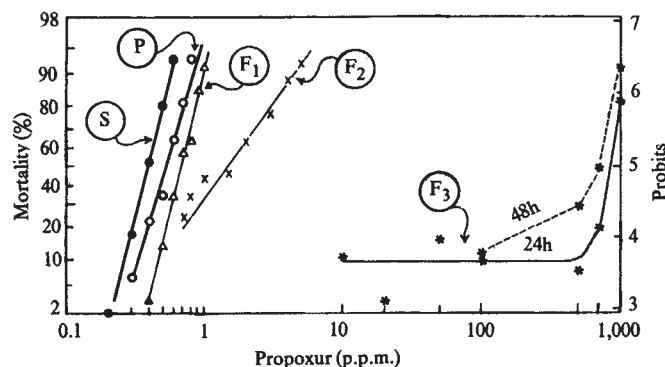


Fig. 1 Dose/mortality relationships of larvae of susceptible (S), El Salvador (P) and propoxur-selected (F_1 , F_2 , and F_3) populations of *Anopheles albimanus*.

subsequent fourteen generations, exhibited only a $3 \times$ increase in larval LC_{50} and $3.5 \times$ increase in adult LC_{50} (ref. 4).

We recently studied populations of this mosquito from El Salvador, where it is resistant to both DDT and dieldrin^{3,8,9} and has also been exposed to parathion, methyl parathion, malathion, trichlorfon, thiometon, 'Azodrin' (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate), and DDT. In El Salvador and elsewhere in Central America, *A. albimanus* is often found breeding in or near cotton fields¹⁰ which are heavily treated with these insecticides. As many as thirty applications of insecticides during the 6 month cotton growing season are not unusual (unpublished information from various sources including the El Salvador Department of Agriculture).

Eggs were obtained from adults collected on June 16, 1970, at Hacienda Melara, Department of La Libertad, El Salvador (given by Dr S. G. Breeland) and a vigorous colony was established in our laboratory. The larvae were fed on a mixture of Purina laboratory chow and yeast and the colony was maintained in controlled conditions of 26°–27° C and 60% relative humidity. The adults were fed on rabbits at the initial stages of this work and later on white mice. Larval and adult susceptibility tests were carried out as previously described¹¹. In the adult tests glass-fibre filter paper was substituted for cellulose paper as the latter is known to reduce variability with carbamates¹². The larval LC_{50} values obtained (Table 1) indicate increases in tolerance to propoxur, carbaryl, malathion, parathion and methyl parathion in the range of $1.1 \times$ to $3.4 \times$ compared with a mixed susceptible strain of Haiti/Panama origin.

When we subjected the El Salvador strain to larval selection pressure by propoxur at the 90–95% level of mortality, the

larval LC_{50} rose only gradually at first, that is, from the parental 0.53 p.p.m. to 0.68 p.p.m. in the F_1 generation, and to 1.5 p.p.m. in the F_2 (Fig. 1). A change in slope of the dosage-mortality line of the F_2 generation from 3.3 in P_1 to 1.4 was more indicative of impending resistance. Selection for a third generation resulted in a marked increase in resistance to propoxur. Concentrations of 10–500 p.p.m. produced 24 h mortalities of from 3 to 15%; 1,000 p.p.m. resulted in only 81% mortality. If, at the end of the 24 h exposure, the larvae were held in clean water for an additional 24 h, only slight evidence of delayed mortality was observed. In these conditions, concentrations of 100, 500, 700 and 1,000 p.p.m. produced 11, 28, 48 and 91% mortality, respectively (Fig. 1).

To characterize the cross-resistance pattern of the strain, susceptibility tests were carried out on an unselected generation derived from the F_3 , referred to as F_{3+1} . There was no detectable difference in susceptibility of F_3 and F_{3+1} larvae to propoxur. The tests indicated that resistance extends also to the carbamate carbaryl at a very high level ($74.8 \times$), and further it involves several organophosphorus insecticides, namely parathion ($35.5 \times$), methyl parathion ($36.9 \times$), malathion ($20 \times$), and fenitrothion ($9.6 \times$) (see Table 1). A lower level of resistance to paraoxon (approximately $5 \times$) was found, and little or no resistance to dichlorvos and fenthion. The above levels of resistance are interesting because they appear to be approximately proportional to the pre-selection levels found in the parental field strain. Laboratory selection by propoxur therefore had the effect of magnifying the low levels of organophosphorus resistance which existed in the field population. Selection also increased the level of DDT resistance from the initial $4.6 \times$ to $20 \times$, but had no effect on dieldrin resistance.

Until biochemical tests are completed we cannot know the precise mechanisms responsible for this resistance. Evidence for physiological resistance, however, is readily apparent in the results so far obtained with synergists. The effects of piperonyl butoxide, triphenyl phosphate (TPP), S,S,S-tributyl phosphorothioate (TBPT), and 1,1-bis-(*p*-chlorophenyl) ethanol (DMC) were studied by pretreating the resistant larvae with a sublethal dose of synergist 4 h before treatment with the insecticide (Table 2). Synergists were used at 10 p.p.m. except for TBPT which was used at 20 p.p.m. Piperonyl butoxide caused no increase in mortality with 100 p.p.m. propoxur, but with 500 and 700 p.p.m. it produced a marked increase. The absence of synergism at 100 p.p.m. of propoxur indicates that at this dosage a sufficient amount of detoxication enzyme remained uninhibited and was able to cope with the insecticide. At doses of 700 p.p.m. and above, however, the detoxication capacity of larvae was rapidly depleted. In contrast, carbaryl synergism occurs at lower dosages of insecticide. Synergism of carbamates by piperonyl butoxide suggests that microsomal mixed function oxidases are involved in resistance¹³. Further evidence of this is the effect of piperonyl butoxide on the toxicity of the phosphorothioates parathion, methyl parathion, fenitrothion, and fenthion. As expected, a strong degree of antagonism towards these was observed, indicating the inhibition of oxidative enzymes involved in the $P=S$ to $P=O$ activation step. With the phosphates dichlorvos and paraoxon no detectable effect was noted (Table 2). Because of the high degree of resistance to parathion ($35.5 \times$), some synergism of paraoxon might also be expected. That none was observed indicates that resistance involves an attack on the thionate before its conversion to the phosphate and/or non-oxidative degradation of the phosphate. In this respect, the low level of synergism obtained on paraoxon by TBPT, an inhibitor of desethylation¹⁴, may be significant. The synergism of malathion by TPP would also suggest the presence of carboxylesterase detoxication enzymes¹⁵.

A limited number of tests on adults revealed that resistance to propoxur is considerably lower in this stage than in larvae. The LC_{50} value of the parental adults ($0.47 \mu\text{g}/\text{cm}^2$) rose only $4.3 \times$ in the F_{3+1} generation ($2.04 \mu\text{g}/\text{cm}^2$). It is therefore

Table 1 Response to Various Insecticides in Susceptible, Parental, and Propoxur-selected Strains of *Anopheles albimanus*

Compound	24 h LC_{50} (p.p.m.)				
	Haiti/ Panama susceptible	El Salvador P_1	RR *	Propoxur-selected F_{3+1}	RR *
Propoxur	0.39	0.53	1.4	> 100.0	> 100.0
Carbaryl	0.89	1.02	1.1	66.6	74.8
Malathion	0.085	0.25	2.9	1.7	20.0
Parathion	0.0031	0.0098	3.2	0.11	35.5
Methyl para- thion	0.0065	0.022	3.4	0.24	36.9
Fenthion	0.023	0.026	1.1	0.027	1.2
Fenitrothion	0.025	0.045	1.8	0.24	9.6
Dichlorvos	0.11	0.11	1.0	0.21	1.9
DDT	0.01	0.046	4.6	0.20	20.0
Dieldrin	0.003 †	1.37	456.7	1.30	433.3

* Resistance ratio = $\frac{LC_{50} P_1 \text{ or } F_{3+1} \text{ strain}}{LC_{50} \text{ susceptible strain}}$

† LC_{50} of susceptible Gorgas strain.

likely that selection for reduced cuticular penetration may also account for a portion of the observed resistance in larvae.

We expect that studies in progress in this laboratory will provide the necessary qualitative and quantitative information on the nature of the resistance factors involved. We believe, however, that the information so far obtained on resistance to propoxur and cross-resistance to fenitrothion is of major importance in mosquito control and in malaria eradication strategies in particular, since both compounds are considered as front line substitutes against DDT and dieldrin resistant anophelines.

Table 2 Effect of Synergists on the Toxicity of Various Compounds to Propoxur-selected *Anopheles albimanus*

Insecticide and dose (p.p.m.)	Synergist and dose (p.p.m.)	Percentage mortality	
		Insecticide alone	Insecticide + synergist
Propoxur 100	pb*	10	9
Propoxur 500	pb	10	7
Propoxur 700	pb	10	19
Carbaryl 30	pb	10	13
Carbaryl 50	pb	10	25
Malathion 2	TPP†	10	56
Parathion 0.175	pb	10	94
Methyl parathion 0.4	pb	10	93
Fenitrothion 0.3	pb	10	76
Fenthion 0.05	pb	10	95
Dichlorvos 0.2	pb	10	40
Paraoxon 0.2	pb	10	0
Paraoxon 1	pb	10	80
Paraoxon 0.2	TBPT‡	20	0
Paraoxon 1	TBPT	20	80
DDT 1	DMC§	10	76

* Piperonyl butoxide.

† Triphenyl phosphate.

‡ Tributyl phosphorothioate.

§ 1,1-bis-(p-chlorophenyl) ethanol.

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V. ARIARATNAM
G. P. GEORGHIOU

Department of Entomology,
University of California,
Riverside,
California 92502

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Acceptability of Daily *l*-Tetramisole by Pound Dogs infected with *Dirofilaria immitis*

THIENPONT *et al.*¹ reported on the anti-helminthic potency of a new synthetic drug named tetramisole which, when given in a single dose, was effective against many of the intestinal and pulmonary nematodes of fourteen mammalian species. In the absence of any information on the host's reaction to tetramisole given for 30 to 40 days, an experiment was designed with the primary objective of testing the acceptance of daily doses of the drug by dogs from pounds. A secondary objective was to detect any therapeutic activity against adult *Dirofilaria immitis*.

Three pound dogs, weighing 40–50 pounds and aged 1–2 yr, were used. Each had a *D. immitis* microfilaraemia, with pre-treatment concentrations of (A) 83, (B) 105 and (C) 5 microfilariae/20 mm³ of blood respectively. Each was given one 50 mg tablet of tetramisole, directly or in a meatball, daily from Monday to Friday for 3 weeks, after which the dose was increased to one 100 mg tablet for the next 3 weeks. Four days after the last 100 mg dose, each dog was given a single dose at the rate of 9 mg per pound body weight.

The dogs were killed by intravenous injection of barbiturates 16 days after the last administration of drug and body cavities and viscera examined for worms. The great vessel leading to the heart was clamped off and the heart and lungs removed as a block. All chambers of the heart, the pulmonary artery, as well as all the dissectible branches of the arterial tree were opened in the search for adult heartworms. A gross examination for pathological changes was performed on all organs and principal tissues.

There were no unusual problems of drug acceptability with any of the three tetramisole regimens used. Dead worms were found in all dogs, often in the second order pulmonary arteries. All worms removed were placed in tissue culture fluid so that any movement could be observed, but none was seen. The microfilariae concentrations just before necropsy were: (A) 29; (B) 0; and (C) 0/20 m³ of blood.

In the conditions of this limited experiment, there is clear evidence that tetramisole can be given to pound dogs at low concentrations for extended periods. Indications against the therapeutic use of tetramisole as a microfilaricide and as an adulticide were not observed clinically. More study is needed to determine the minimum effective concentration of tetramisole to destroy both microfilariae and adults of *D. immitis*.

G. S. TULLOCH
R. A. ANDERSON

Veterinary Sciences Division,
USAF School of Aerospace Medicine,
Brooks Air Force Base, Texas 78235

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Rational Design of Degradable Insecticides

I HAVE used a sterically based hypothesis of the mode of action of aryl insecticides, including DDT¹, to predict possible structures of new compounds, the synthesis² of which has been directed at finding insecticides with low mammalian toxicity and which, because of their inherent chemical instability, would readily degrade. Insecticidal activity has been studied

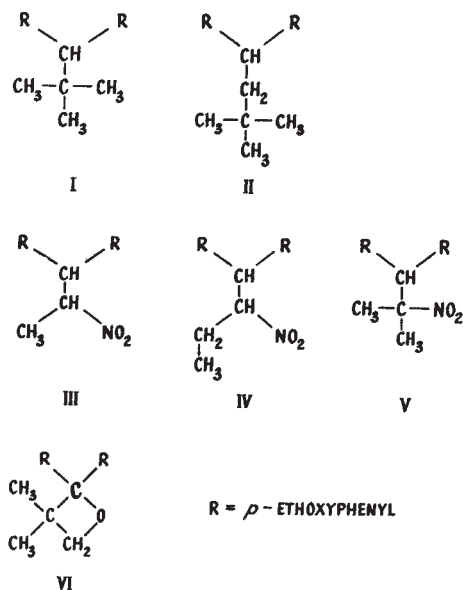


Fig. 1 Three series of non-halogenated insecticides designed from a theoretical model. In the nitroalkane and oxetane series the compounds containing two or at least one *p*-ethoxyphenyl-substituent exhibit a higher insecticidal activity than their *p*-chlorophenyl-analogues.

in susceptible and resistant housefly and investigations have been made of acute mammalian toxicity and chemical *in vitro* degradation.

The model consists essentially of a size limited "molecular wedge" which relates the physiological properties of the insecticides to their ability to keep the channel of Na⁺ ions open³ during an action potential pulse in a nerve membrane. The broad base of the "wedge" (for example, the *p*-chlorophenyl rings of DDT) was assumed to lock the whole structure by charge transfer complex formation into the protein matrix of the nerve membrane. This usually requires two phenyl

rings substituted with electron donor groups. Chlorine atoms in active structures are not essential, as demonstrated by the activity of several series of new insecticides (Fig. 1 and Table 1) which do not contain halogens. These were tested for insecticidal activity in a DDT susceptible strain of housefly (WHO/IN/Musca d./1) by the method described before.

An alternative to chlorine substitution in the phenyl rings comprising the base of the "wedge" was found in alkoxyphenyl derivatives. The *p*-ethoxy substituent, for example, has optimum electron donating properties within the model size limitation. Using this group the investigation of new structures centred on the design of the apex of the "wedge". In the original hypothesis, the chemical structure of the apex was only relevant with respect to size and chemical stability, and on this principle three types of compounds, branched hydrocarbons, nitroalkanes and dimethyl oxetanes, were selected and prepared (Fig. 1). The three series illustrate the importance of relating the size of the group at the apex to constructed steric three dimensional atomic models. We propose that the limit of the projected maximum van der Waals diameter (0.6 nm ± 0.05) of this group determines the activity or inactivity of the compounds. In neopentane I (Fig. 1, Table 1) which is related to the less active dimethoxy analogue XV (Table 1)^{4,5}, the shift of the *t*-butyl group in its homologue, the neohexane II (Fig. 1, Table 1), does not alter its downward projection and the same insecticidal activity is retained.

The three nitroalkanes III, IV and V (Fig. 1) also demonstrate the importance of the design. The *p*-chlorophenyl analogues of the first two are insecticides, 'Prolan' and 'Bulan'⁶, and the inactivity of 1-*o*-ethoxyphenyl-1-*p*-ethoxyphenyl-2-nitropropane⁷, can be explained by its wrong dimensional fit into the described model, which suggested the high activity of the *bis-p*-ethoxyphenyl-compounds and the activity of the previously unknown nitroalkane isomer V (Fig. 1, Table 1). The best illustration of this design, however, is in the oxetane series VI-X (Table 1). The oxetane heterocyclic ring was selected on the basis of the construction of three dimensional atomic models and the projections of the groups. Fig. 2 shows the effect of the change of conformation of the methyl groups on the oxetanes from the 3,3-dimethyloxetane VII

Table 1 Structure-Activity Relationship of New Insecticides their Synergism and Mammalian Toxicity

No.	Compound	LD ₅₀ <i>Musca d.</i> (μg/insect)	LD ₅₀ <i>Musca d.</i> * + synergist (μg/insect)	Synergistic ratio	LD ₅₀ mouse (mg/kg)	LD ₅₀ mouse <i>Musca d.</i> (mg/kg)
I	1,1- <i>bis</i> -(<i>p</i> -ethoxyphenyl)-2,2-dimethylpropane	1.92	0.32	5.5	5,200	54
II	1,1- <i>bis</i> -(<i>p</i> -ethoxyphenyl)-3,3-dimethylbutane	2.00	0.36	5.5		
III	1,1- <i>bis</i> -(<i>p</i> -ethoxyphenyl)-2-nitropropane	0.48	0.065	7.4	1,150	48
IV	1,1- <i>bis</i> -(<i>p</i> -ethoxyphenyl)-2-nitrobutane	0.55	0.061	9.0	1,160; 980 †	42; 326 †
V	1,1- <i>bis</i> -(<i>p</i> -ethoxyphenyl)-2-methyl-2-nitropropane	1.01	0.053	19.6		
VI	2,2- <i>bis</i> -(<i>p</i> -ethoxyphenyl)-3,3-dimethyl oxetane	0.52	0.010	52	1,200	46
VII	2,2- <i>bis</i> -(<i>p</i> -chlorophenyl)-3,3-dimethyl oxetane	1.27	0.66	1.9	3,500	55
VIII	2,2- <i>bis</i> -(<i>p</i> -chlorophenyl)- <i>trans</i> -3,4-dimethyl oxetane	> 100	> 100			
IX	2,2- <i>bis</i> -(<i>p</i> -methoxyphenyl)-3,3-dimethyl oxetane	5.3	0.25	21.0		
X	2,2- <i>bis</i> -(<i>p</i> - <i>n</i> -propoxyphenyl)-3,3-dimethyl oxetane	5.3	0.53	10.0		
XI	2- <i>p</i> -ethoxyphenyl-2- <i>p</i> -methoxyphenyl-3,3-dimethyl oxetane	1.43	0.086	17.9		
XII	2,2- <i>bis</i> -(<i>p</i> -ethoxyphenyl)-3,3,4-trimethyl oxetane	> 100				
XIII	1- <i>p</i> -ethoxyphenyl-1- <i>p</i> -methoxyphenyl-2-nitropropane	0.25	0.014	17.9		
XIV	1,1- <i>bis</i> -(<i>p</i> -methoxyphenyl)-2-nitropropane	> 20				
XV	1,1- <i>bis</i> -(<i>p</i> -methoxyphenyl)-2,2-dimethylpropane ^{4,5}	3.2	1.70	1.9		
XVI	1,1- <i>bis</i> -(<i>p</i> -chlorophenyl)-2-nitrobutane (Bulan)	1.69	1.78	0		
XVII	1,1- <i>bis</i> -(<i>p</i> -chlorophenyl)-2,2,2-trichloroethane (DDT)	0.24	0.25	0	570	47

* 'Sesamex' 1% solution, 0.5 ul. added with each dose of insecticide.

† Toxicity test using 2 : 1 'Sesamex' to insecticide ratio.

(Table 1) to the *trans*-3,4-dimethyl- VIII, or 3,3,4-trimethyl oxetane, XII which alters the downward projection and the diameter of the end group. According to the theoretical model the projected maximum diameter of the last two derivatives is too large and the compounds are inactive. The dimensionally correct structures are highly active insecticides. The potentiated 1,1-*bis-p*-ethoxyphenyl derivative VI (Table 1) was twenty-five times as active as DDT against the housefly.

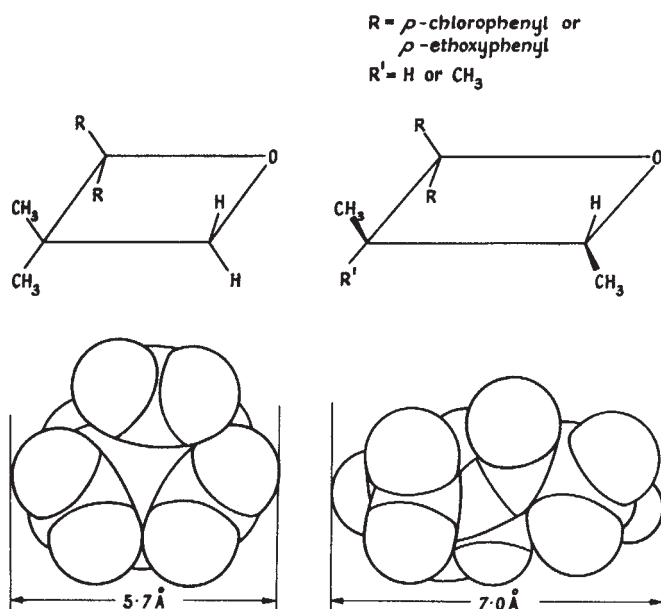


Fig. 2 The effect of conformation on activity of 2,2-*bis*-phenyl-dimethyl oxetane insecticides. The downward projection of steric atomic models with phenyl rings removed shows the change in diameter (in Angstrom units) of the end groups for the insecticidally active 3,3-dimethyl oxetanes and inactive 3,3,4-trimethyl or *trans*-3,4-dimethyl oxetanes.

A major degradative mechanism of organic insecticides in insects and mammals is the action of microsomal mixed function oxidase^{8,9}. The susceptibility of the new alkoxyphenyl structures to such attack was predicted from their chemical properties, and demonstrated by the strong potentiation of activity (Table 1), even in susceptible houseflies, by inhibitors of this enzyme system¹⁰. Potentiation was tested by applying 0.5 μ l. of a 1% solution of 'Sesamex' (2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxundecane) in acetone immediately after

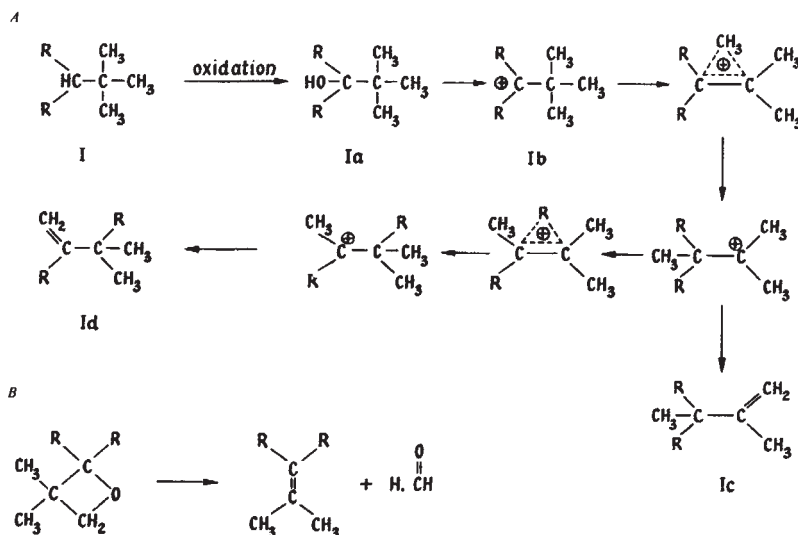
each dose of insecticide applied to the dorsum of the thorax of the housefly.

The ethoxyphenyl neopentane I (Fig. 3) is readily oxidized *in vitro* at the benzylic carbon and the neopentyl carbonium ion Ib formed from the labile 1-carbinol Ia rearranges spontaneously by the illustrated mechanism to a single product, the biologically inactive olefin Id. When R was phenyl or *p*-chlorophenyl, small amounts of olefin Ic were also isolated. Similar degradation should take place in the case of the related neohexane homologue II (Fig. 1). The major modes of biological degradation may parallel these *in vitro* transformations, and this is supported by the finding^{8,11} that microsomal oxidation converts DDT by hydroxylation at the benzylic carbon to 1,1-*bis-p*-chlorophenyl-2,2,2-trichloroethanol. This metabolite, however, in contrast to carbinol Ia (Fig. 3), is stable.

In vitro degradation of all the insecticidal 2,2-dimethyl oxetanes VI, VII, IX, X, and XI (Table 1) was a major factor in their selection for synthesis, which takes place spontaneously (Fig. 3), yielding the inactive 1,1-diaryl-2,2-dimethylethylene and formaldehyde, and can be controlled by the addition or removal of free radical initiators¹². When adsorbed on non-biological surfaces the oxetanes degrade rapidly. A sample of 1,1-*bis*-(*p*-chlorophenyl)-2,2-dimethyl ethylene co-chromatographed with the 1,1-*bis*-(*p*-chlorophenyl)-3,3-dimethyl oxetane VII, on a deactivated preparative TLC silica gel plate using benzene as the eluent giving bands with R_fs 0.18 and 0.42 respectively. When extracted after 24 h exposure, both bands yielded only the 1,1-*bis*-(*p*-chlorophenyl) dimethyl ethylene. This rapid surface degradation of the oxetane insecticides contrasts with their stability in tests on solid samples or solutions of the compounds lasting several months, disproving the reported¹³ inherent instability of some 3,3-diaryl oxetanes which could not be isolated but only detected spectrometrically in solution.

Acute mammalian toxicity was tested for several new compounds in each series (Table 1) using Swiss albino female mice of average weight 20 g. Compounds were administered intraperitoneally in olive oil and toxic effects were observed for 5 days. Five animals were used per concentration and the median lethal dose for each compound was calculated. The last column of Table 1 shows the ratios of mammalian to insecticidal toxicity in identical units (mg/kg; tested average 50 flies/gm). Even though the compounds were applied in different ways—some by topical application, others by intraperitoneal injection—a comparison of the values for the two species demonstrates an approximate fifty-fold safety margin in the acute toxicity in favour of the mammals, which is constant for different structures. The ratio of toxicities using the alkoxyphenyl nitrobutane IV (Table 1) with added

Fig. 3 Chemical degradation in two series of the new insecticides. A, The phenyl neopentyl structures are degraded to inactive olefins Ic and Id after hydroxylation to carbinol Ia. This rearrangement takes place at room temperature in the presence of even weak Lewis acids such as LiCl. B, The dimethyl oxetanes are degraded spontaneously when adsorbed on surfaces to formaldehyde and inactive 1,1-diaryl-2,2-dimethylethylenes. This degradation can be controlled by the addition of free radical initiators. All degradation products were identified by proton NMR, mass spectra and elemental analyses.



synergist demonstrates the selectivity of the synergist to insects and also the advantage of the potentiation in improving mammalian-insect toxicity ratio. For DDT XVII (Table 1) the ratio cannot be increased as its insecticidal activity cannot be potentiated. Although tests were carried out on only two species it is reasonable to conclude that for these compounds there is no intrinsic acute mammalian toxicity inherent in the chlorine substitution and that toxicity is potentiated by microsomal oxidation inhibitors predominantly in the insect.

mammalian safety margins, non-halogenated, degradable structures and activity against resistant pests, outweigh the economic disadvantages of the compounds. Beneficial properties are sometimes coupled with decreased persistence and need for potentiation which may make the new compounds unacceptable under present agricultural and public-health practice of pest control.

Mammalian toxicity tests were carried out in part at the Department of Pharmacology, University of Melbourne, and

Table 2 Mortality Data for DDT Resistant Strains of Housefly

Numbers from Table 1 of 1,1-bis-(p-ethoxy)- or (p-chlorophenyl)-compounds	Turramurra *	WHT (P) †	F ₃	Housefly strain and LD ₅₀ values (µg/insect)			DDT F ₃₇
				F ₁₀	F ₄₁	F ₅₆	
I -2,2-dimethylpropane	3.2	3.3	3.6	4.7	> 200		
I + 'Sesamex'	0.32 (0.1%)				4.2 (0.1%)		
III -2-nitropropane	0.80	0.32	0.86	2.74	—	0.33	
III + 'Sesamex'	0.06 (0.1%)	0.09 (1.0%)	—	0.19 (0.5%)	—	0.04 (1.0%)	0.11 (0.1%)
IV 3-nitrobutane	0.86						
VII -3,3-dimethyl oxetane	2.38						
VII + 'Sesamex'	0.66 (0.1%)						
XVII -2,2,2-trichloroethane (DDT)	> 200	37.3	76.5	> 200	> 200		
DDT + 'Sesamex'	> 200 (1.0%)				> 200 (1.0%)		

* Activity of compounds against a DDT resistant strain kept under DDT pressure and † against a field strain resistant to DDT, selected with compounds I, III and DDT for F number of generations. The selection with each compound was carried out on approximately 2,000 flies at 75% mortality level for each generation. Values in brackets are solution concentrations of 'Sesamex' synergist applied with each dose of the insecticide. All test values are for female flies. Mortality data were determined 48 h after topical application of insecticide and synergist in an acetone solution to two day old female flies fed on sugar only diet. Mortalities were compared with zero mortality in identically treated controls.

The decrease in the degree of oxidative degradation of p-chlorophenyl analogues such as DDT (revealed comparing the synergism of oxetanes VII and VI of Table 1) probably accounts for their persistence and that of their metabolites in the environment.

The most important factor which would account for the general low mammalian toxicity of this type of insecticide is the negative coefficient of insect mortality to temperature, which was also demonstrated for the new structures¹⁴. In warm blooded animals this effect would decrease the "locking" of the aryl structures into nerve membranes and diminish the nerve ion-flux disturbances attributed to this action.

The genetically developed enzyme DDT-dehydrochlorinase, which is a major factor in the build-up of resistance¹⁵ to insecticides containing the 2,2,2-trichloroethane structural group, obviously cannot affect the nonhalogenated compounds listed in Table 1. The new insecticides had high activity against a field strain of the housefly resistant to DDT (Table 2). In a laboratory study using the 1,1-bis-(p-ethoxyphenyl)-2,2-dimethylpropane I and 2-nitropropane analogue III (Table 2) the parent generation (WHT-P) was a heterozygous field strain of the housefly with considerable resistance to DDT. For the three compounds tested in each generation approximately 2,000 flies were selected at a 75% level. A sample of female flies was then treated and the mortalities were counted 48 h later. The parent strain rapidly developed a complete resistance to DDT; resistance to the dimethylpropane I developed more slowly, while the nitropropane III gave inconsistent results and the initially developed resistance could not be maintained. Although the use of the 'Sesamex' synergist controlled the resistance to the new compounds, additional modes of resistance to DDT-type insecticides are now recognized¹⁶ and further studies are required to determine their development by selection with the listed compounds.

Although positive results were obtained in the rational

design of insecticides the question remains whether higher the Commonwealth Serum Laboratories, Melbourne. I thank Dr E. Shipp for supervising the entomological tests and Mr R. Eibl for technical assistance.

G. HOLAN

Division of Applied Chemistry,
CSIRO,
P.O. Box 4331,
Melbourne, Victoria 3001

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Photomimetic Effect of Acetylcholine on Morphogenesis in *Trichoderma*

BIETH *et al.*¹ have demonstrated that synthetic photochromic agents can effect control of acetylcholinesterase activity *in vitro*, and suggest that photomorphogenetic processes are mediated by similar systems. Jaffe² has shown that acetylcholine mimics red light in some plant photomorphogenetic processes in which phytochrome is considered to be the photo-acceptor. He also found increased efflux of acetylcholine from roots following red light treatment.

Various fungi have a photomorphogenetic pigment system which differs from the phytochrome system of higher plants insofar as the photoacceptor absorbs blue instead of red light^{3,4}.

Sporulation (conidiation) is induced at the growing edge of 1 day old cultures of the imperfect fungus *Trichoderma viride* Pers. ex Fries by minuscule quantities of blue light; the half maximum response is reached with 6.6×10^{-10} Einsteins/cm² at 447 nm³. Evidence with inhibitors suggests a requirement for nucleic acid synthesis for morphogenesis as sporulation can be selectively suppressed by some base analogues while only slightly affecting growth⁵.

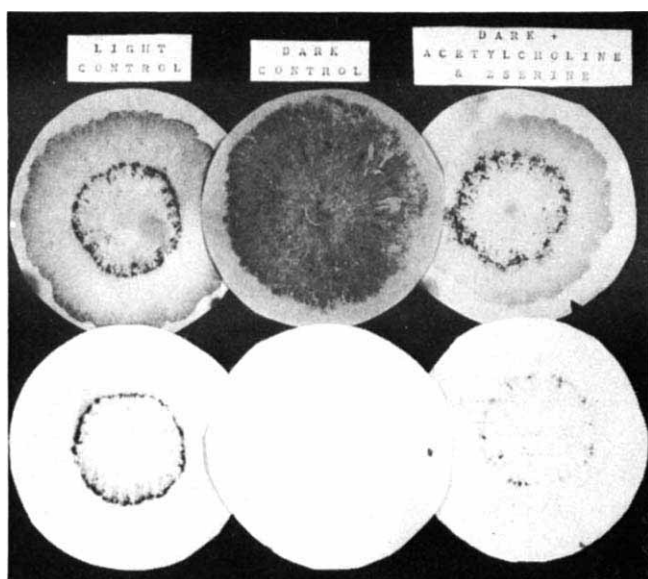


Fig. 1 Dark induction of sporulation (conidiation) in *Trichoderma* by acetylcholine and eserine. An agar block with mycelium of *Trichoderma viride* Pers. ex Fries was cultured for 34 h on one circle of Whatman No. 50 hardened filter paper, soaked with 2.3 ml. of double strength Difco potato dextrose broth, in sterile conditions at 24° C. The filter paper circles were then transferred to a second dish containing a 7 cm circle of Whatman No. 1 filter paper, soaked with 1.0 ml. of the above medium with or without the addition of 10^{-4} M acetylcholine (BDH) and 5×10^{-6} M eserine (Calbiochem) final concentrations. The light controls were then illuminated for 3 min with fluorescent lights. All other culturing procedures were performed with a red safelight. Bottom row: fixed in 80% ethanol 32 h after transferring; top row: fixed as above, dried and then stained with cotton blue to show mycelia as well as spores.

We wished to ascertain whether the blue light-induced morphogenetic response could be mediated through an acetylcholine type response, in a similar manner as that of the higher systems.

Agar blocks of plant fungal mycelium were planted on filter paper soaked with medium and cultured for 30–34 h. The filter papers were then transferred to Petri dishes containing

fresh media with the experimental additives. Control cultures were illuminated for 3 min to induce sporulation. Detailed methods are described in ref. 5.

Preliminary experiments showed that acetylcholine alone could not induce sporulation in the dark under a wide range of concentrations (10^{-5} to 10^{-1} M). In the presence of the acetylcholinesterase inhibitor eserine (physostigmine) it was possible to induce sporulation, mimicking photoinduction in conditions which slightly inhibited growth (Fig. 1). Harsh physical and nutritional conditions such as desiccation or temporary starvation can induce a diffuse sporulation quite different from the distinct ring of spores induced by light or by acetylcholine. The acetylcholine antagonist curare suppressed light-induced sporulation, as would be expected from our preliminary findings, but it inhibited growth as well.

The ranges of effective acetylcholine and eserine concentrations inducing sporulation were found to be narrow (Fig. 2).

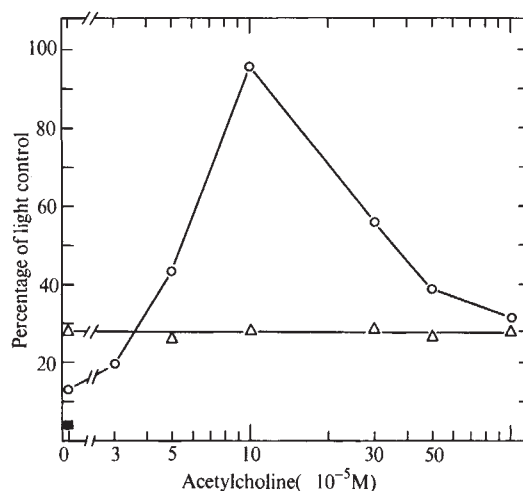


Fig. 2 Effect of acetylcholine and eserine concentrations on dark sporulation of *Trichoderma*. The experiments were performed as in Fig. 1. After ethanol fixation and drying, the spores were washed off in distilled water with a rubber policeman and counted microscopically in a Levy-Hausser corpuscle counting chamber. About 3×10^3 spores were contained in a light control spore ring. Results with 10^{-5} M eserine were similar to those with 3×10^{-6} M eserine shown above. ○, 5×10^{-6} M eserine; △, 3×10^{-6} M eserine; ■, dark control.

Higher plants contain a blue light absorbing photomorphogenetic pigment system as well as the red light absorbing phytochrome. This blue light system has a similar action spectrum to that of *Trichoderma*. In some plants the direction of morphogenesis is sometimes controlled by an interplay of the two pigment systems⁶. If both systems act through acetylcholine some rethinking of the modes of action of each system will be necessary.

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J. GRESSEL
LYNN STRAUSBAUGH
E. GALUN

Department of Plant Genetics,
Weizmann Institute of Science,
Rehovot

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Proof of Two Distinct Enhancement Effects by Blue Light on Oxygen Uptake in *Chlorella*

MANY authors have reported an enhancement of oxygen uptake in *Chlorella* by blue light¹⁻⁴. The pigment responsible has not been definitely established, but may well be a flavin⁵. The mechanism of the enhanced oxygen uptake is also uncertain.

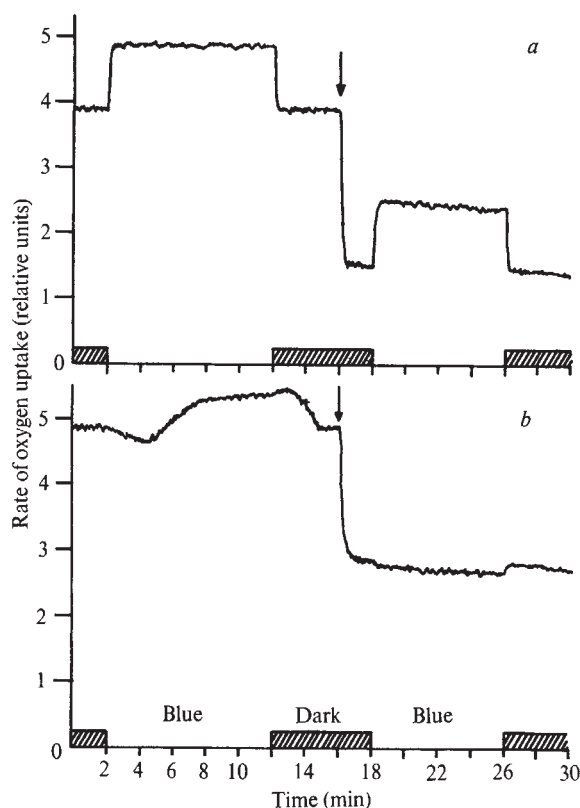


Fig. 1 The effect of blue light and cyanide poisoning on the respiration rate of two *Chlorella* strains. *a*, Colourless *Chlorella* mutant "G-27" in the presence of 3×10^{-5} M FMN (the curve has been corrected for uptake due to the auto-oxidation of FMN²); *b*, *C. pyrenoidosa* chick treated with 5×10^{-6} M DCMU. Cyanide (final concentration 2×10^{-4} M) was added to the preparations at the time indicated by the arrows. The enhancement was stopped in *b*, but not in *a* (pH=6.6 in all cases). Note the immediate onset of the enhancement in *a*, in contrast to *b*.

The enhanced respiratory activity has a respiratory quotient (RQ) of 1, and the same temperature dependence as dark respiration^{5,6}; the cyanide sensitivities of the light stimulated oxygen uptake and of that part of glucose (dark) respiration which is inhibited by cyanide are identical⁵. It has been suggested^{5,6} that the effect is due to a stimulation of normal

respiratory pathways, possibly caused by an increased level of substrate (for example, a release of stored carbohydrate). Laudenbach and Pirson⁷ investigated the effect of red and blue light on the carbohydrate content of the cells, and concluded that the effects of light on respiration are secondary and that a more direct effect might be a change in permeability of the plastid membrane.

On the other hand, Schmid and Schwarze⁴ have reported oxygen uptake enhanced by blue light in a colourless *Chlorella* mutant, which they attribute to an FMN-dependent amino-acid oxidase. Crude enzyme extracts of this oxidase showed enhanced oxygen uptake in blue light, implying that blue light acts directly to increase oxygen uptake. With this amino-acid oxidase Schmid and Schwarze could account for the fact (reported in most of the above mentioned publications) that starvation of the cells for many hours before illumination gave an increased blue effect. They reasoned that in starvation the cells start hydrolysing their own protein, giving a higher concentration of free amino-acids available for direct oxidation. The authors assumed the enhancement effect observed in their mutant was the same as that reported by other groups, although one would not expect an RQ of 1 for protein oxidation. No inhibitor, temperature dependence or other studies were reported. We have found a second *C. pyrenoidosa* mutant ("G-27", described by Bendix and Allan⁸) with this FMN-dependent amino-acid oxidase activity⁹.

Table 1 Distinguishing Features of the Two Types of Blue Light-stimulated Oxygen Uptake in *Chlorella*

Enhancement effect	Type I	Type II
Time of appearance	Delayed 6-10 min	Within 5 s
Cyanide sensitivity	97% inhibited at concentration of 2×10^{-4} M (ref. 5)	Unaffected by concentration of 2×10^{-2} M
Respiratory quotient	1	?
Pigment responsible	Possibly FMN	Probably FMN ⁹
Probable mechanism	Stimulation of normal endogenous respiration and electron transport chain	FMN-dependent amino-acid oxidase
References cited	1-7	4, 9

We have now shown that this blue light phenomenon is different from that found in the normal (photosynthetic) *Chlorella* strain (Fig. 1). In the light of this we re-examined other reports of blue light enhancement effects in *Chlorella* and found that both effects had been observed, but not distinguished. We therefore wish to alert other investigators to this possible pitfall. Two easily checked, distinguishing features are the time course of the effects and their cyanide sensitivities. In DCMU-treated cells of the normal, photosynthetic strain, blue light causes an initial inhibition of oxygen uptake, which is followed 6-10 min later by an enhancement. This enhancement is virtually eliminated by 2×10^{-4} M cyanide (Fig. 1b), and is the same effect reported by Ried and further described by Pickett. The blue light-enhanced oxygen uptake in the "G-27" mutant, however, appears within 5 s (Fig. 1a) and is not inhibited even by 2×10^{-2} M cyanide.

For ease of identification we suggest the delayed, cyanide-sensitive effect be labelled "type I" and the immediate, cyanide-insensitive effect "type II". The properties of each are summarized in Table 1, together with the cited references allocated according to which effect they seem to describe. In the future authors should make clear which effect is being studied.

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DAVID F. SARGENT
C. P. S. TAYLOR

Biophysics Department,
University of Western Ontario,
Ontario

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Possible Ball Court at La Venta, Mexico

LA VENTA, one of the earliest Olmec sites, is located on an island in the Tonala River which forms the boundary between the Mexican states of Veracruz and Tabasco (18° 10' N, 94° 5' W). The site is believed to have been built and maintained during the period from 1000 BC to 600 BC or even later¹, primarily as a ceremonial or religious enclave². For years, no ball court, usually an important feature of sacred Mesoamerican precincts, had been found at La Venta³.

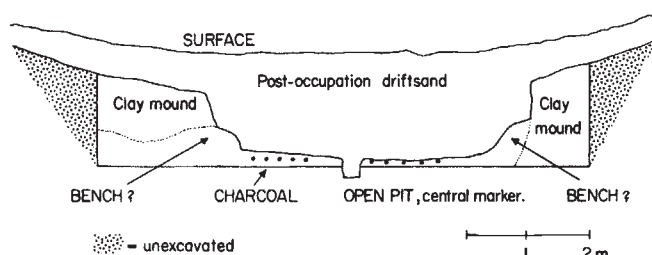


Fig. 1 Cross section through suspected ball court at La Venta, Mexico.

By 1963, at least 267 ball courts had been identified in Mesoamerica⁴. The Mesoamerican ball game is said to have originated in the La Venta culture and to have spread to the Maya and other Mesoamerican cultures⁵. An early stela found on the Veracruz Gulf Coast (the Matisse stela) depicts what is thought to be a ball player with hands upraised in front of his face⁶. Monument thirty-four at San Lorenzo, another Olmec site, also probably represents a ball player⁷, while the helmeted colossal Olmec stone heads possibly represent champions of the ball game⁸. At San Lorenzo, ball player figurines have been found in the levels of the San Lorenzo phase which has been placed by radiocarbon dating to a range from 1150 to 900 BC (private communication from M. Coe). It has even been suggested that the ball court heroes were all politicians⁹.

A structural complex recently discovered at La Venta, which may be a ball court, consists of two parallel mounds at the southern end of the "Stirling group", each about 14 m wide. That on the west is 55 m long and the other 39 m long¹⁰, which suggests that the ball court was of the open ended type and thus stylistically consistent with other early courts.

A sample of charcoal from the uppermost clay construction layers of the court floor (Fig. 1) has been dated (UCLA-1630) at 2710 years (760 BC) using the newer half-life of $5,730 \pm 30$ yr. This radiocarbon date is consistent with various others obtained from the site^{1,11-13}.

The earliest court so far found in the lowland Maya area is probably that at Copan which is the earliest in a superimposed series of three courts and which possibly belongs to the Early Classic period (AD 300-600). This was a comparatively narrow open court, smoothly paved with low slanting ramps on either side and possibly with plaster markers^{14,15}.

UCLA-1630 seems to be the first radiocarbon date for a ball court, previous dating having been based on methods of varying degrees of accuracy, from ceramic association to assessment of architectural style and hieroglyphic inscriptions⁴. Another suspected ball court of perhaps similar antiquity is that at the Olmec site of San Lorenzo, constructed of earth and clay fill and built during the Palangana phase. This phase appears to have cross-ties to Mamón and to Drucker's La Venta material which places it into the 600-400 BC range (Coe, private communication).

The date of 760 BC would make the feature at La Venta the oldest ball court in Mesoamerica. The oldest definitely identified ball court seems to be that at Monte Alban and attributed to the Monte Alban Period II, which began at about the end of the third century BC, when Maya-like features, including stelae engraved with hieroglyphics, appeared at Monte Alban⁶. The detailed features of this court are unknown, but later Alban and Zapotec ball courts are noteworthy in being fully enclosed and in having two niches in opposite diagonal interior angles of the court instead of rings or other devices⁶. The dating of Monte Alban has been subject to numerous uncertainties¹⁶, and most recently excavations at Lambityeco, near Monte Alban, by John Paddock are said to raise implications that Monte Alban had fallen several centuries earlier than had been believed, leaving a serious gap in dating sequences¹⁷.

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LILLIAN WORTHING WYSHAK
RAINER BERGER

Departments of Anthropology and History
and Institute of Geophysics,
University of California,
Los Angeles

JOHN A. GRAHAM
ROBERT F. HEIZER

Department of Anthropology,
University of California,
Berkeley

Received March 8; revised April 5, 1971.

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Do Intracellular Concentrations of Potassium or Sodium Regulate the Strength of the Heart Beat?

CERTAIN "inotropic" responses in which the strength of the heart beat increases are accompanied by a loss of potassium from the cells and a simultaneous gain of sodium¹. The question of immediate interest is whether these changes of cellular ion content and of contractile force—if indeed they are always thus associated—reflect a mechanism which controls the heart twitch or whether they are incidental. We have set out to re-examine this question, much debated in the past but not yet entirely resolved².

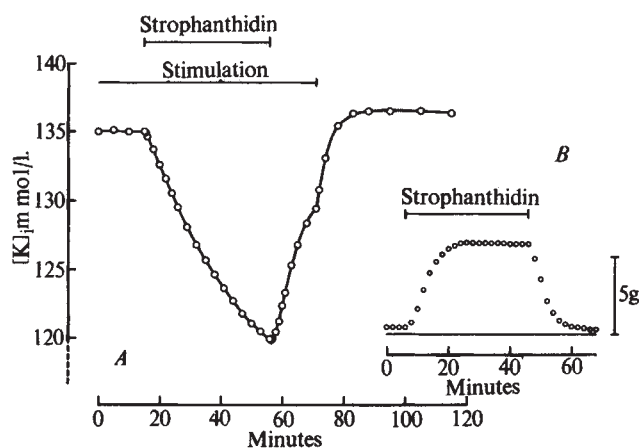


Fig. 1 Effects of 3-acetyl strophanthidin (5×10^{-7} M) on cellular potassium movements (A) and twitch tension (B). External medium: 0.5 mM $[Ca]_o$, 2 mM $[K]_o$, and isethionate substituted for chloride. A, Ventricle stimulated for 15 min immediately before start of the experiment, and thereafter for the time indicated by bar (rate 20 min^{-1}). Ordinate: Concentration in cell water. B, Peak twitch tension (plotted at 2 min intervals) developed by a ventricle stimulated (at 20 min^{-1}) throughout. (For details of technique, see ref. 14.)

Two parallel series of experiments were made to examine (i) the changes in the twitch tension developed by frog ventricles (*Rana pipiens*) during various inotropic responses, and (ii) the associated net movements of potassium between the heart and perfusion fluid (measured essentially as described in ref. 3, using the improved experimental procedure developed for the study of calcium fluxes⁴). We were thus able to confirm and extend some earlier results by showing that many, though not all, of the inotropic responses examined are accompanied by a net efflux of potassium ions from the heart cells, followed by a net influx during recovery from the inotropic effect. Potassium movements of this kind were quite marked during responses resulting from (i) changes in the stimulus rate ("staircase" phenomena), (ii) reduction of the external potassium concentration, $[K]_o$, and (iii) application of the cardiac steroid 3-acetyl strophanthidin (see magnitude of the potassium movements in Figs. 1, 3 and 4). Further, all these results were essentially unchanged when the normal perfusate containing 118 mM Cl was replaced by one in which chloride was substituted by larger

anions such as isethionate or ethyl sulphate, which are known to penetrate the cell membrane only slowly^{5,6}. While this excludes any appreciable contribution of anion movements, the possibility remains that the cation exchange, that is, cellular potassium for external sodium, is essential to the mechanism underlying the inotropic responses. Indeed, two hypotheses invoked to interpret the above and other related results are based on this idea, the earlier correlating strength of contraction with the level of potassium (refs. 1, 7, but compare 8), the later with that of sodium inside the heart cells⁹. In particular, it has been proposed^{9,10}, and widely accepted^{11,12}, that changes in cellular sodium concentration are linked with those of "activator" calcium and thus help to regulate contractile activity.

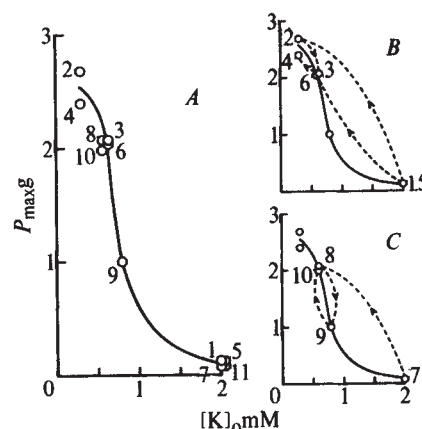


Fig. 2 Steady state twitch heights in media of different $[K]_o$. 0.5 mM $[Ca]_o$, isethionate substituted for chloride. Stimulus rate 20 min^{-1} . A, Dose-action curve, peak tension P_{max} , versus $[K]_o$, summarizing results of an experiment; numbers indicate sequence of changes in $[K]_o$. B, and C, Schematic representation of the different pathways (in the same experiment) leading from tension level in 2 mM $[K]_o$ to that in 0.6 mM $[K]_o$: cf. 1→2→3 and 5→6 in (B), 7→8 and 7→8→9→10 in (C). (Note also the two pathways to tension level in 0.3 mM $[K]_o$: 1→2 and 1→2→3→4 in (B).)

In analysing these ideas further, the subsequent experiments were made in Cl-free media, with isethionate as the substitute anion, to ensure that movements of sodium of essentially the same size, but opposite direction, accompanied the potassium movements which we determined. (Of course, the equivalence is not exact because of the movements of other ions, notably calcium. The change in the intracellular ion balance, however, as produced, for example, by the known calcium movements in similar conditions¹³, is too small to affect the validity of the above assumption and of the conclusions to be derived later.)

Three sets of results emerged which are difficult to reconcile with the view that contraction is sensitive to changes in level of cell potassium or sodium. (1) During some of the inotropic responses, twitch tension and level of cellular potassium, $[K]_i$, altered with markedly differing time courses, the changes of tension being usually more rapid than those of $[K]_i$. Fig. 1 illustrates this difference by the effects obtained with a submaximal dose of acetyl strophanthidin (5×10^{-7} M; maximum twitch tension was obtained with concentrations greater than 10^{-6} M in otherwise similar experiments). Twitch tension rose to a new steady level within 15–20 min of the application of the drug (Fig. 1B), whereas the level of $[K]_i$ continued to decrease, though at a gradually declining rate, for much longer (> 40 min). The later part of Fig. 1 shows the

recovery from the drug action: the tension declined to the original low level (Fig. 1*B*) and the tissue reabsorbed potassium after withdrawal of the drug, and subsequent cessation of stimulation (Fig. 1*A*).

Experiments in which the effects of low $[K]_o$ were examined in the same way as described for acetyl strophanthidin yielded closely similar results—a rapid increase of tension (within 10–20 min) to a steady high level, associated with a progressive loss of potassium (lasting >40 min).

(2) Steady twitch tensions recorded in a low $[K]_o$ medium were not related to intracellular potassium (or sodium) concentrations, which could be adjusted by pretreatment of the ventricles with various low potassium fluids. Fig. 2*A–C* illustrates the steady state tensions in an experiment in which a ventricle was exposed to fluids of various $[K]_o$ for 10–20 min (2 mM being the level in “normal” fluids). As is seen, twitch heights in a 0.6 mM $[K]_o$ medium were little different whether recorded after the concentration step from 2 to 0.6 mM $[K]_o$ or after the more complex sequences which included exposure of the ventricle to either 0.3 or 0.8 mM $[K]_o$. (Compare sequences 5→6 and 1→2→3, and sequences 7→8 and 7→8→9→10 in Fig. 2*B* and *C* respectively). By contrast, potassium release from the cells markedly depended on the sequence of concentration change, being, for example, 7.2 mmol/l. cell water (± 0.7 , s.e. of five determinations) after the change from 2 to 0.6 mM $[K]_o$, as against 16.3 mmol/l. (± 1.2 , s.e. of five determinations) after the change through the level of 0.3 mM $[K]_o$. (These results were obtained from ten experiments made in similar conditions to those of Fig. 2.)

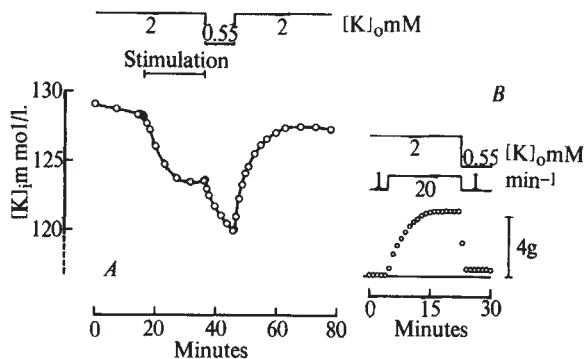


Fig. 3 “Ascending” and “descending” staircase in media of different $[K]_o$. 1.2 mM $[Ca]_o$, isethionate substituted for chloride. *A*, Cellular potassium movements associated with periods of quiescence and stimulation (rate 20 min^{-1}) in 2 mM $[K]_o$, and with a subsequent period of quiescence, first in 0.55 mM and then in 2 mM $[K]_o$. *B*, Peak tension of twitches (plotted at 1 min intervals) in response to a high stimulus rate (20 min^{-1}) in 2 mM $[K]_o$, and to a subsequent low rate (1 min^{-1}) in 0.55 mM $[K]_o$.

It should also be mentioned that pretreatment of heart cells with very low potassium fluids ($<0.5 \text{ mM } [K]_o$) sometimes affected the subsequent twitch tension which, however, became weaker rather than stronger, possibly as the result of “fatigue” (compare tension levels 2 and 4 in 0.3 mM $[K]_o$ of Fig. 2, and also the results of Fig. 4*B*).

(3) Well defined conditions exist in which changes in twitch tension were associated with potassium and sodium movements in directions opposite to those predicted by any of the hypotheses outlined here. For example, the weakening of heart twitches during a period of reduced stimulus rate (“descending” staircase), which is usually associated with potassium uptake (and sodium loss), also occurred when heart cells were made to lose potassium (and gain sodium) by exposure to a low $[K]_o$ medium during this period. Indeed, the decline of twitch tension so obtained was quite similar to that of a normal “descending” staircase, except that the final steady level of tension was slightly higher

in the low potassium fluid (Fig. 3*B*). The loss of potassium during this tension decline was considerable, being comparable with that associated with the build up of tension during the “ascending” staircase (compare cellular K loss during stimulation period in Fig. 3*A*) and was followed by a net gain of this ion by the cells during the final recovery period in normal $[K]_o$ medium.

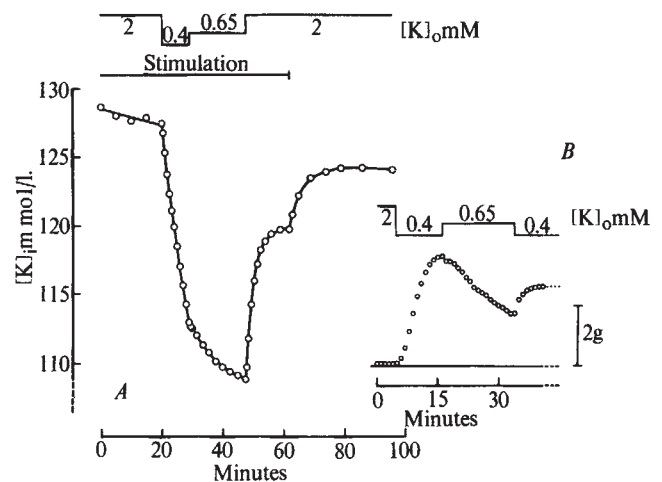


Fig. 4 Effects of several step changes of $[K]_o$ on cellular potassium movements (*A*) and twitch tension (*B*). External medium: 0.5 mM $[Ca]_o$, isethionate substituted for chloride. *A*, Ventricle stimulated as in Fig. 1. *B*, Peak tension of twitches (plotted at 1 min intervals) during continuous stimulation (rate 20 min^{-1}) in media of different $[K]_o$ as indicated. Final dotted line indicates that on return to 0.4 mM $[K]_o$ tension normally attained a plateau, though often of reduced height (compare discussion in connexion with Fig. 2).

Fig. 4 is another illustration of the concurrence of tension changes with ion movements in the “wrong” direction. Here the responses to a series of changes in $[K]_o$ were followed, first, to a step decrease, from 2 to 0.4 mM, which caused tension to rise, then to a smaller step up, from 0.4 to 0.65 mM, which caused tension to fall (Fig. 4*B*). The cellular potassium release (Fig. 4*A*) associated with the first period was in accordance with the hypotheses tested, but not its continuation during the second period, during which the direction of tension change reversed. The final sections of Fig. 4*A* and *B* show the essentially unimpaired responsiveness of the tissue to further tests, even after large and rapid losses of potassium. Thus, (a) potassium was taken up again after the re-establishment of normal $[K]_o$, and cessation of stimulation, respectively (Fig. 4*A*), and (b) the tension rose once more on again reducing $[K]_o$ (Fig. 4*B*).

In conclusion, although it is still conceivable that cellular potassium for sodium exchanges contribute in regulating the strength of the heart beat, this contribution is unlikely to be at all substantial. More probably, the principal factor common to many inotropic responses resides in the cell membrane, in as yet unknown processes which control the magnitude of cellular calcium fluxes.

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D. C. GADSBY
R. NIEDERGERKE
S. PAGE

Department of Biophysics,
University College, London

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Acquisition of Human Antigens by *Schistosoma mansoni* during Cultivation *in vitro*

Schistosoma mansoni may live in the portal circulation of man for many years. In the rhesus monkey, established adult worms persist even though their presence stimulates a powerful host immunity against reinfection with cercariae¹. We have suggested that this concomitant immunity may be explained if the adult worms possess host antigens²; such an immunological disguise would allow these established worms to evade the efferent component of the host's immune response which kills new invading forms lacking the disguise. The existence of these host antigens at the surface of the worms has been demonstrated by transferring worms, grown to maturity in mice, directly into the portal circulation of rhesus monkeys immunized against mouse erythrocytes. Such mouse worms are rapidly destroyed by an antibody-mediated response directed against sites on the worms' surface; mouse worms survive well on transfer to normal monkeys³.

These mouse antigens are not permanently expressed in the worm, and could no longer be detected using this system when mouse worms had been in the circulation of normal monkeys for 7 days. Nevertheless, there is good evidence that these antigens are not merely gross contaminants of host origin; they seem to be firmly bound to or incorporated into the surface membrane of the worm³⁻⁵. They do not seem to be mouse IgG immunoglobulin, neither are they Forsmann antigen; but they are antigenically identical to other components of the mouse erythrocyte membrane⁴. They are not present in cercariae but are partly expressed in 7 day schistosomula and fully expressed in 15 day schistosomula⁵.

The origin and mode of acquisition of these antigens are unknown. We believe that they are truly of host origin and, by some means, they become incorporated into the surface structure of the schistosome. An alternative hypothesis, favoured by Capron and his colleagues⁶, suggests that these antigens are synthesized by the schistosomes and mimic those of the host. It is postulated that the schistosome possesses a range of codes for these antigens and specific selection for a given mimicking antigen takes place when the worm enters a particular host.

We have recently studied the acquisition of host antigens by schistosomula during cultivation *in vitro*. Such a system is much more susceptible to detailed analysis than an *in vivo* system, and it may eventually be possible to select between the hypotheses outlined above. Because *S. mansoni* is primarily a parasite of man, and much is known of human

erythrocyte antigens, we grew the schistosomes in human blood.

Schistosomula of a Puerto Rican strain of *S. mansoni*⁷ were collected by allowing cercariae to penetrate specially prepared isolated mouse skin; they were then cultured *in vitro* for 15 days (J. A. C. and S. R. S., in preparation). The culture medium consisted of equal volumes of fresh human or monkey serum and Earle's balanced salt solution, lactalbumin hydrolysate at a final concentration of 0.25%, 1% washed human or monkey erythrocytes and antibiotics. The medium was gassed with 8% CO₂ in air to give a pH of 7.4. Incubation was at 37° C and the medium was replaced at intervals of 3-4 days, except that the original erythrocyte inoculum was retained. Two ml. of this medium, in a Leighton tube, supported the growth of 200-250 schistosomula. At 15 days, the schistosomula had shown satisfactory development according to the criteria of Clegg⁸; indeed they were more advanced than schistosomula grown *in vivo* in mice for the same period.

Meanwhile, rhesus monkeys were immunized with various types of washed human erythrocytes in Freund's complete adjuvant³; control monkeys were injected with adjuvant alone or not treated. Three weeks after primary immunization, the monkeys received a booster intraperitoneal injection of washed erythrocytes. One week later, the 15 day schistosomula were washed in Earle's solution containing 1% monkey serum and transferred to immunized and control monkeys by cannulation of the mesenteric veins⁵. Each monkey received a carefully counted number of schistosomula; the actual numbers transferred varied between 165 and 247 in the first experiment and 105 and 128 in the second. Four or 5 weeks after transfer, when any surviving worms would have reached maturity, the monkeys were killed and perfused and the adult worms were recovered⁷.

Table 1 Acquisition of Human Host Antigens by Schistosomes *in vitro*

Monkey No.	Immunization	Erythrocytes in culture system	Worm recovery 5 weeks later (%)
601	FCA	Monkey	67
608	Nil	Monkey	79
602	FCA	AB—	73
607	Nil	AB—	45
603	Anti-AB—	Monkey	103
604	Anti-AB—	Monkey	96
605	Anti-AB—	AB—	3
606	Anti-AB—	AB—	5

FCA, immunization with Freund's complete adjuvant alone.

The human blood used in the first experiment was of type AB Rh—; control cultures were maintained in normal rhesus monkey blood. Table 1 summarizes the plan and the results of this experiment. Control monkeys accepted schistosomula grown in either human or monkey blood with equal readiness and survival rates were high. Anti-AB— monkeys accepted worms grown in monkey blood but almost completely destroyed those cultured in AB— blood. Evidently, these schistosomula had acquired human antigens during their cultivation *in vitro* and these served as targets for the immune response of the anti-human monkeys.

The second experiment was designed to determine whether blood group specific antigens were among those acquired by the schistosomula. Two bloods differing in respect of several major antigens were chosen: A, DE, N—, Fy(a+); and B, dē, N+, Fy(a—). Rhesus monkeys were immunized with one or other type of washed erythrocytes in Freund's complete adjuvant followed by a booster dose intraperitoneally; control

monkeys were injected with Freund's complete adjuvant alone. Schistosomula were cultivated for 15 days in media based on these bloods and were then washed and transferred into rhesus monkeys according to the scheme shown in Table 2. Five weeks later, the surviving adult worms were recovered.

Table 2 Specific Blood Group Substances as Host Antigens

Group	Monkey No.	Immunization	Erythrocytes in culture system	Worm recovery 5 weeks later (%)
Control	618	FCA	A+	76
	619	FCA	A+	45
	624	FCA	B-	73
	625	FCA	B-	85
Homologous	614	Anti-A+	A+	3
	615	Anti-A+	A+	3
	622	Anti-B-	B-	1
	623	Anti-B-	B-	3
Heterologous	620	Anti-A+	B-	14
	621	Anti-A+	B-	23
	616	Anti-B-	A+	3
	617	Anti-B-	A+	33

FCA, immunization with Freund's complete adjuvant alone.

Students *t* test was carried out after arc sin transformation of the data. Control versus homologous: $P < 0.001$; control versus heterologous: $P < 0.01$; homologous versus heterologous: $P < 0.05$

When the transfer was homologous, "A+ worms" into anti-A+ monkeys and "B- worms" into anti-B- monkeys, almost all the schistosomula were destroyed. With heterologous transfer, "A+" worms into anti-B- monkeys and vice versa survival rates were more variable. Certainly, significantly fewer worms survived than in the control monkeys, but more survived than in the homologous transfers. We interpret this result as meaning that the schistosomula acquired, *in vitro*, antigens which were common to the two erythrocyte types but also, to some degree, specific blood group antigens.

We may conclude that the schistosomula of *S. mansoni* can acquire human antigens and that these antigens can be acquired during cultivation *in vitro*. Thus the *in vitro* system appears to offer a valid approach to further studies. Human antigens common to A+ and B- erythrocytes are acquired by schistosomula in culture and there is some evidence that blood group specific antigens are expressed as well. If this last finding can be confirmed, we believe that it supports the view that host antigens are truly of host origin and are not synthesized by the worm. We doubt that the selective synthesis of human blood group specific antigens is within the capabilities of a trematode parasite, even one as well adapted as *S. mansoni*.

We thank Mr John Harris for determining the blood group specificities and Mr Kenneth Gammage for assistance.

J. A. CLEGG
S. R. SMITHERS

National Institute for Medical Research,
London NW7 1AA

R. J. TERRY

Brunel University,
London W3 9BX

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Biphasic and Reversible Adaptation of Goldfish to Alcohol

GOLDFISH are well suited to the study of the effects of alcohol on behaviour. The concentration of alcohol in their blood rapidly approximates the concentration of alcohol in the water in which they swim¹. Thus the study of tolerance, for example, is not complicated by such variables as rate of alcohol absorption. We have studied certain responses of goldfish as a function of time in an alcohol solution, to learn whether tolerance to alcohol develops, and whether it persists after return to water.

Twenty-four goldfish (*Carassius auratus*), 3–5 cm long, were kept in 100 l. tanks containing tap water. At the start of each session, twelve were randomly assigned to an experimental group and twelve to a control group. The fish were transferred to smaller tanks containing 0.8% ethanol solution (experimental fish) or water (control fish), remaining in the respective solution, one fish per tank, for periods of 0.5 h to 42 h.

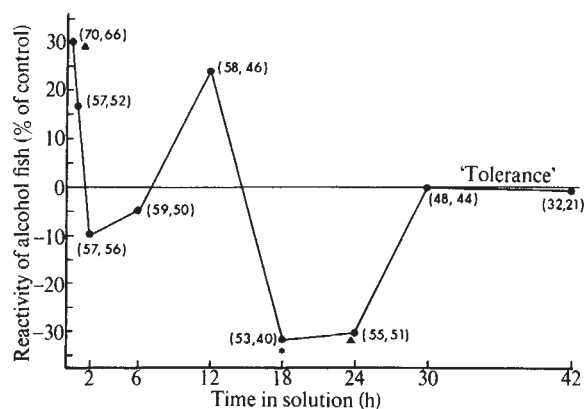


Fig. 1 Biphasic effects of alcohol on goldfish responding to an aversive stimulus after immersion in an 0.8% alcohol solution for varying periods. Each point represents the difference in the mean value of experimental fish from the mean value of concurrently run control fish, expressed as a percentage of the control value. Numbers in parenthesis represent, respectively, the experimental N and control N for each immersion period. Significant differences from control means are indicated by an asterisk ($P < 0.05$) and triangle ($P < 0.01$). The 12 h response was not significant ($P > 0.10$).

We measured an escape reaction to an aversive stimulus. The experimental apparatus consisted of a trough 118 cm long, 3 cm wide, 4 cm deep. At one end was a "start box" 6 cm long, 3 cm wide, separated from the remainder by a trap door. A high intensity light faced the start box, opposite the trap door. For each trial a fish was placed in the start box for 30 s, the light was turned on for 3 s, and the trap door was lifted, allowing the fish to escape from the light. Response was meas-

ured as distance, in cm, the fish swam from the light in 5 s. The unresponsive fish or those that moved less than 5 cm were excluded from the analysis so that the numbers of fish in each group are unequal. Each fish was used only once so that they should not become "practised". Experimental and control fish were tested simultaneously, the trough containing the pretest-period fluid. Alcohol in water and blood was assayed by gas-liquid chromatography. Equilibration occurred within 60 min.

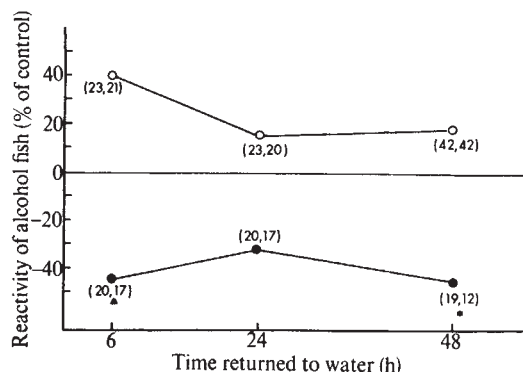


Fig. 2 Fish "tolerant" to alcohol after 42 h of immersion in an 0.8% alcohol solution returned to water for periods of 6, 24, and 48 h. Fish reimmersed in alcohol for 0.5 h continued to show an excitatory response (○). Fish immersed in alcohol for 24 h still showed a depressed response (●). Each point represents the difference in mean value of experimental fish from concurrently run control fish, expressed as a percentage of the control value. Significant differences from control means are indicated by an asterisk ($P < 0.02$) and solid triangles ($P < 0.01$). Numbers in parenthesis represent, respectively, the experimental N and control N.

Fish in alcohol for 0.5 h were significantly more reactive than control fish (Fig. 1). They also were more reactive after 1 h. Fish in alcohol for 2 and 6 h were a little less reactive. They were more reactive after 12 h. After 18 and 24 h, experimental fish were much less reactive. After this "depressed" phase, the groups responded similarly after 30 and 42 h of immersion.

These results indicate that during adaptation to an 0.8% alcohol solution, when the alcohol blood level rises from zero to 800 mg/100 ml, in 1 h, the goldfish pass through three phases of activity. They are hyper-reactive for about 1 h, then hypo-reactive after about 18 h, and lastly they become "tolerant" after about 30 h.

A second study was conducted to learn whether tolerance persists after return to water. Fish were kept in an 0.8% alcohol solution or water for 42 h as described. Experimental and control fish were then returned to separate water-filled tanks for 6, 24 and 48 h. After each interval, experimental fish were returned to individual tanks containing an 0.8% alcohol solution and controls were returned to individual tanks containing water. After 0.5 h, they were tested in the aversive stimulus trough. Another group of fish were immersed in alcohol or water for 24 h before testing. These periods were chosen because in the original experiment alcohol fish were maximally excited and maximally depressed at 0.5 h and 24 h, respectively. If tolerance persisted after return to water, reimmersion in alcohol could be expected to (1) abolish the excitatory phase at 0.5 h, (2) abolish the depressed phase at 24 h, or (3) do both. This did not occur. Tolerant fish, after return to water, still showed an excitatory phase after 0.5 h in alcohol and a depressed phase after 24 h in alcohol (Fig. 2).

As noted, fish occasionally failed to swim away from the light. The percentage of nonresponders in the experimental and control groups differed markedly. 9% of the alcohol group and 23% of the control group ($\chi^2 = 49.57$; $P < 0.001$)

failed to respond. The difference existed each time fish were tested (Table 1). To investigate this more deeply, we repeated the experiment with one change: two fish, instead of one, were placed in the small containers. Tested after 42 h of immersion (Table 1), the percentage of nonresponders was the same in both groups. A tank mate, in short, had the same effect as alcohol in reducing the number of nonresponders.

Table 1 Comparison of Nonresponders in Alcohol and Control Groups

Time in solution (h)	N	Alcohol group Nonresponders (%)	N	Controls Nonresponders (%)
0.5	75	7	75	12
1	62	8	62	16
2	62	8	62	10
6	61	3 *	62	19
12	62	6 *	62	26
18	60	12 *	61	34
24	60	8	61	16
30	56	14	62	29
42	41	22 *	47	55
42 (with two fish)	19	21	24	21

"Nonresponders" refers to fish that swam less than 5 cm from a start box in an aversive stimulus trough, the stimulus being a high intensity light. All fish were kept in single-fish tanks containing either alcohol or water, except in the final trial, when two fish were kept in each tank.

* Significant difference between alcohol and control fish ($P < 0.01$).

It seems, therefore, that goldfish can become adapted to alcohol and that the initial response is biphasic. Tolerance of alcohol is rapidly extinguished if acclimated fish are returned to water. The excitatory phase may result from a rapidly rising blood alcohol level, but the depressed phase and subsequent tolerance apparently reflect duration of exposure, since blood alcohol concentration is constant after 1 h of immersion. Finally, "social isolation" of goldfish apparently reduces responsivity to an aversive stimulus, and alcohol cancels this effect.

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DONALD W. GOODWIN
CAROL P. DOWD
SAMUEL B. GUZE

Department of Psychiatry,
Washington University School of Medicine,
4940 Audubon Avenue,
St Louis, Missouri 63110

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Larval Development and Metamorphosis of *Acanthaster planci* (Asteroidea)

LITTLE is known of the factors influencing survival during the planktonic phase of *Acanthaster planci* (Linnaeus), the crown of thorns starfish, apart from an observation of fish eating

newly released eggs¹. But it is not surprising that this phase has been suggested as the cause of the recent plague of the animal in the Pacific²⁻⁴, for large females release many millions of eggs each spawning season¹ and any factor which slightly reduces the mortality rate during planktonic life and settlement greatly increases recruitment into the starfish populations.

In this study *A. planci* was reared in the laboratory from fertilization to juvenile starfish and a preliminary study has been made of the influence of temperature, salinity, food and substrate on survival and development. Previously, Henderson⁵ and Mortensen⁶ reared *A. planci* to late bipinnaria and brachiolaria stages, respectively.

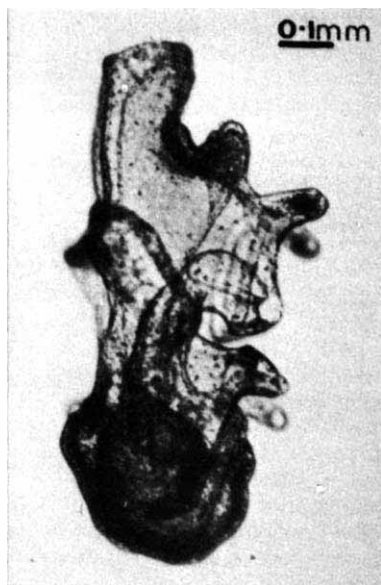


Fig. 1 Lateral view of an early brachiolaria of *Acanthaster planci*.

Mature starfish were collected from a cay 40 miles ENE of Cardwell, North Queensland (146° 51' E, 18° 2' S), on January 22, 1971; larvae were obtained by the method of Henderson⁵. On day 3 the larvae were transferred to 10 l. glass aquaria containing seawater, 33‰ salinity, with 50 IU penicillin G and 0.05 mg streptomycin sulphate/ml. A mixture of unicellular algae was added on days 3 and 9. On day 15 the larvae were transferred from the Fisheries Laboratory, Mourilyan, to James Cook University and placed in glass dishes, about 100 per dish containing 250 ml. of filtered seawater, in a shaker bath operating at forty-five shakes per minute. The larvae were transferred to fresh conditions every few days and unicellular algae added daily. Dishes were maintained at 24°–25° C until day 21, then some were raised to 28°–29° C by day 26.

Bipinnariae developed in the manner described before^{5,6}. After day 23 some larvae developed two brachiolar arms on the pre-oral ciliated band; the pre-oral lobes enlarged, an adhesive disk developed and the posterior portion containing the starfish primordium became enlarged, irregular and brown (Fig. 1). The pre-oral lobe apex then developed dorsal and ventral projections, the latter becoming the third brachiolar arm (Fig. 2). Mortensen's brachiolaria⁶ is similar, but the pre-oral arms are obscure and the primordium is poorly developed.

Late brachiolariae tested substrates with the brachiolar arms and settlement began on day 28. Some remained attached by the adhesive disk throughout metamorphosis; others either failed to attach or detached. Metamorphosis into a five-armed starfish, with absorption of larval structures, took two days. The early starfish were 0.3–0.5 mm diameter and each arm carried a red pigment spot, a primary podium and two pairs of tube feet on its oral surface (Fig. 3). Juvenile

starfish, apparently still feeding on the unicellular algae, grew 0.1–0.2 mm in diameter in 10 days with subdivision of the arms and development of the mouth, spines and ossicles (Fig. 4).

Many larvae failed to reach brachiolaria or metamorphosis and regressed to a ciliated, globular form. Larvae completed development at 28°–29° C, while those remaining at 24°–25° C did not advance beyond early brachiolaria. The mean period from fertilization to starfish was 38 days with a range of 30 to 47 days. In other studies, *A. planci* larvae developed more rapidly, developing to late bipinnaria in 12 days at 27° ± 1° C⁵ and to brachiolaria in 16 days⁶. Thus survival and developmental rate vary markedly within a small temperature range. Development may be more rapid in the field than in this study with a long period at 24°–25° C—Barrier Reef water temperatures are 27°–30° C during the breeding season.

A. planci bipinnariae tolerate abrupt salinity changes from 36‰ to 21‰⁵. Late brachiolariae and metamorphosing forms are much less tolerant and many ruptured when transferred from 33‰ to 35‰ salinity. The ruptures were invariably from the starfish primordium and earlier stages in

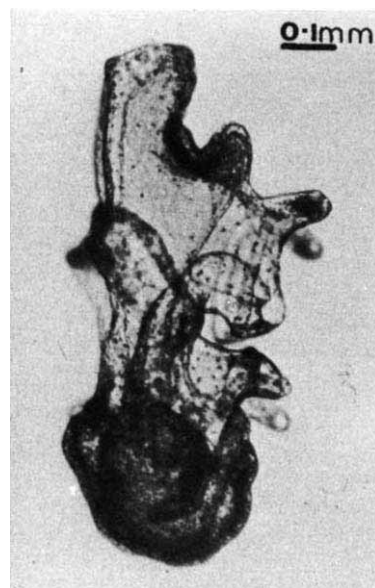


Fig. 2 Lateral view of a late brachiolaria of *Acanthaster planci* showing the three brachiolar arms (1) and adhesive disk (2) (preserved specimen).

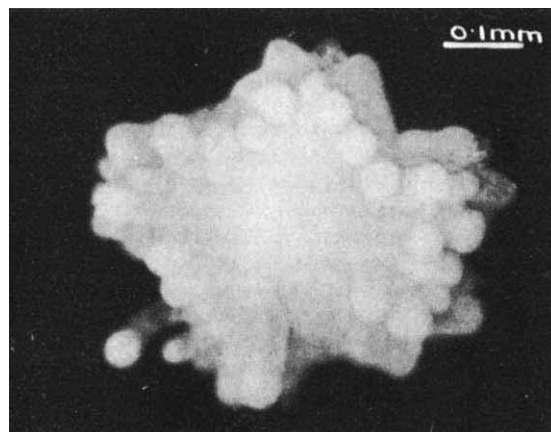


Fig. 3 Oral view of juvenile starfish of *Acanthaster planci* one day after metamorphosis (preserved specimen).

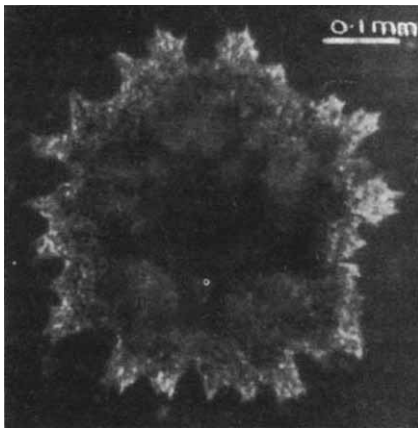


Fig. 4 Aboral view of juvenile starfish of *Acanthaster planci* 14 days after metamorphosis.

the cultures were unaffected. The metamorphosis of ruptured larvae was abnormal.

Larvae fed a mixture of unicellular algae, *Isochrysis galbana*, *Gymnodinium* sp., *Amphidinium* sp., *Cyclotella nana* and *Dunaliella primolecta*, had a predominance of the last alga in their stomachs and some were fed solely with *D. primolecta* from day 22. These larvae metamorphosed at the same time as those on the mixed diet.

Brachiolariae showed a degree of substrate selection at settlement, as observed in other echinoderm larvae⁷. Late brachiolariae kept for up to 14 days in clean, glass dishes without additional substrates failed to metamorphose and some regressed. Simultaneously larvae metamorphosed in other dishes in the presence of encrusting algae and algal detritus. With the exception of a larva which attached to an algal strand on dead coral, *Pocillopora damicornis*, larvae only settled on the bottoms of dishes and there was no significant difference between settlement rates in dishes to which the following substrates were added: macroscopic algae, *Cladophoropsis* sp.; serpulid tubes; fine, siliceous sand; lithothamnion; live *P. damicornis*; *A. planci* spines and tube feet and recently depredated *P. damicornis*.

The period of the *A. planci* life cycle between the earliest starfish, 0.3–0.5 mm diameter, and the smallest, field specimen, 11 mm diameter, collected from coral¹, is still to be studied. This important period includes the transition to adult feeding behaviour.

We thank Mr R. G. Pearson and Mr M. Lamont for their assistance and Professor C. Burdon-Jones for his advice in preparing the manuscript. The work was supported by the Government of Queensland and James Cook University of North Queensland.

J. A. HENDERSON

Department of Primary Industries,
Fisheries Branch,
Harbours and Marine Buildings,
Brisbane, Q 4000

J. S. LUCAS

School of Biological Sciences,
James Cook University of North Queensland,
Box 999 PO,
Townsville, Q 4810

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New Hypothesis of the Cause of Cytoplasmic Incompatibility in *Culex pipiens* L.

If males of one strain of *C. pipiens* are crossed with females of a strain from a different geographical area, the number of offspring may be either normal or small, or there may be none at all¹. These three results have been called compatible, partially compatible and incompatible, respectively. Reciprocal crosses may give the same or different results. For example, strain A males may be fully compatible with strain B females,

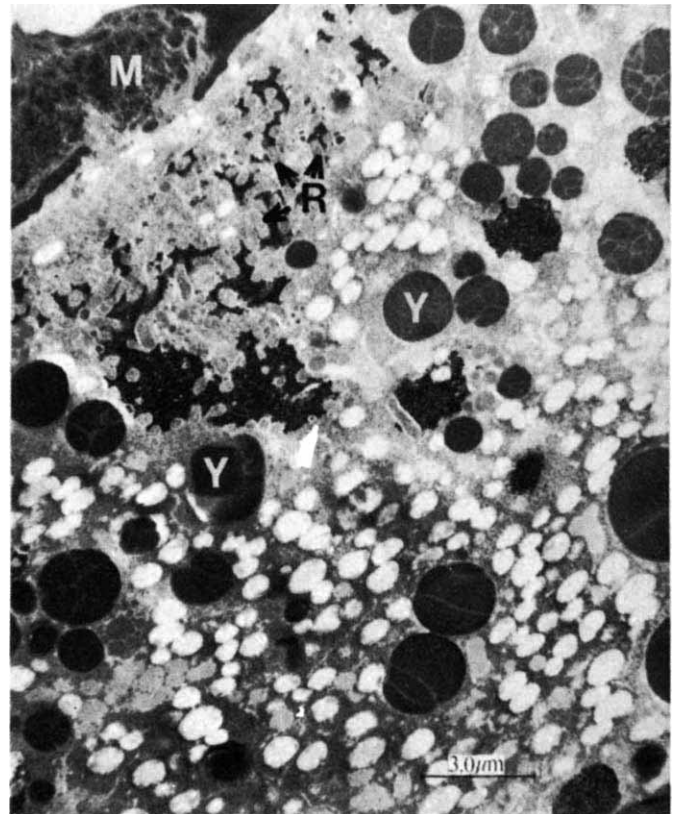


Fig. 1 Rickettsia-like microorganisms (R) in the egg cytoplasm near the micropyle (M). Y, Yolk.

but strain B males may be compatible, incompatible, or only partially compatible, with strain A females.

In an incompatible cross the eggs are fertilized shortly before laying but the male and female nuclei do not unite to form a zygote. The eggs remain haploid and die at various times during embryogenesis². Incompatibility is inherited strictly through the female, as shown by Laven's experiments on genome replacement¹. For this reason the incompatibility is said to be cytoplasmic.

In a partially compatible cross many eggs die, sometimes before fertilization and sometimes after, so that embryonic death cannot be ascribed to haploidy³. It may be very difficult to distinguish between incompatible and partially compatible crosses. True hybrids may be produced in presumably incompatible crosses at a very low frequency, possibly once in 5 or 10,000 incompatible eggs (unpublished results of J. H. Y.).

Although a number of hypotheses have been put forward to explain the inheritance of cytoplasmic incompatibility, none of these offers an adequate explanation and none suggests how it might be caused^{1,4,5}. We wish to propose a new theory based on a cytoplasmically inherited infectious agent. A rickettsia-

like microorganism has been found in substantial numbers in the adults, eggs and embryos from normal and incompatible crosses of *C. pipiens*. These microorganisms are found in the follicles of the ovaries and in recently laid eggs, they are especially abundant near the micropyle (Fig. 1). They are approximately the size of mitochondria but of variable size. In sections prepared for electron microscopy they are seen to be elongate or spherical bodies surrounded by two membranes, and are often seen in association with accumulations of glycogen (Fig. 2). The sperms enter the egg through the micropyle and must pass near or through the mass of microorganisms before they can approach the female nucleus which lies midway down the length of the egg. It is possible that the microorganisms have a deleterious effect on presumably "incompatible" sperm so that they are unable to participate with the oocyte nucleus in the formation of a zygote. The microorganisms appear to be well adapted symbionts of their normal hosts, the particular strains of mosquitoes in which they are normally found, but may be deleterious to sperms of other strains.

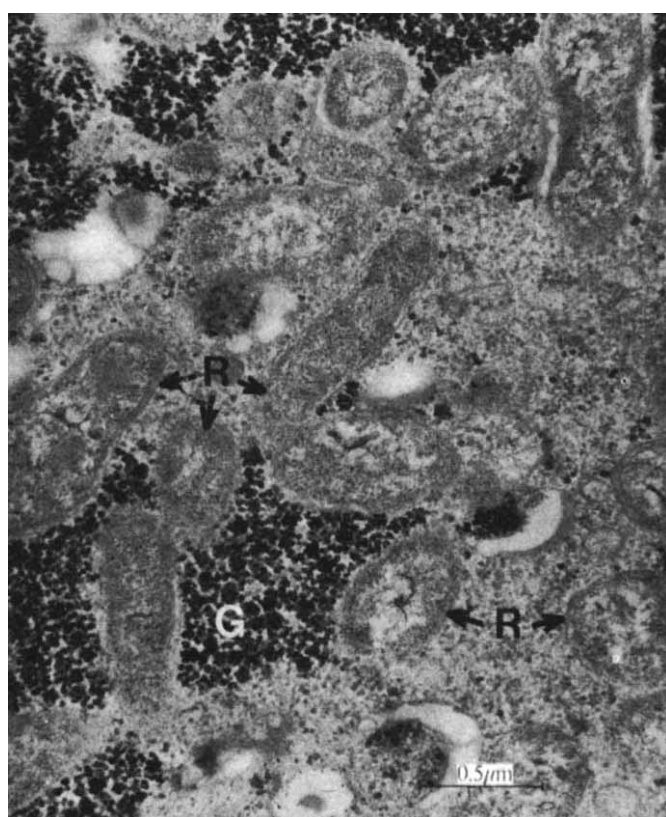


Fig. 2 Enlarged view of the microorganisms in the egg. G, Glycogen; R, rickettsia-like microorganism.

Hertig⁶ described a rickettsia-like microorganism, *Wolbachia pipientis*, from *Culex pipiens* from Boston, Massachusetts. He also found them in specimens from Minnesota and China. Similar microorganisms have been found in other members of the *C. pipiens* complex, in *C.p. quinquefasciatus* Say in Colombia⁷ and in the autogenous form of *C. pipiens* from England⁷ and France⁸. Hertig failed to find them in other species of *Culex* in Massachusetts and Zulueta in other species of mosquitoes in England although he did find them in some species of forest culicines in Colombia. The microorganisms have been found in all the specimens of *C. pipiens* which have been examined.

The presence of coadapted combinations of *C. pipiens* and infectious agents may explain the spectrum of crossing types observed in *C. pipiens*. The microorganisms are consistently present in the reproductive organs of the mosquito throughout

its life cycle and probably kill some follicles and sperm in each generation. If it is assumed that males and females of a strain of *C. pipiens* have the same strain of microorganisms but mosquitoes of different strains have somewhat different microorganisms, then in compatible crosses, the sperm of, for example, strain A which have survived the effect of the microorganisms in the developing testes, are not killed when they enter the eggs of females of strain A which contain the same strain of microorganisms. The sperm fuse with the oocyte nuclei and produce normal larvae. The same sperm from strain A when exposed to a different strain of microorganism, as, for example, in the eggs of strain B, are adversely affected and are prevented from forming normal diploid embryos. In these cases incompatibility is observed and the cross produces no offspring except for an occasional parthenogenetic female. In the reverse cross the effect on the sperm may not be lethal and in this case nonreciprocal incompatibility is the result. Partial compatibility is seen when the microorganisms of a strain of mosquito are not lethal to all sperms from another strain of mosquito. Some sperm survive passage through the mass of microorganisms and unite with the female nuclei to form diploid embryos although some of these succumb to infection later in embryogenesis. Hybrids which survive have gonads which are infected with the maternal strain of microorganism. The gametes they produce have survived the infection, so no effect of the infection is seen in F₂ matings.

The elucidation of the mechanism of cytoplasmic incompatibility in *C. pipiens* is of utmost importance because of its potential use in the control of medically important mosquitoes and because it may clarify our concept of speciation in the *C. pipiens* complex. Our proposed explanation for incompatibility provides a starting point for the solution of these two problems, control and speciation. Much work needs to be done to prove that the microorganisms do in fact cause incompatibility. Infectious agents are known to cause a number of unusual effects in other Dipterans such as sex-ratio distortion⁹, CO₂ sensitivity¹⁰ and male hybrid sterility¹¹. Cytoplasmic incompatibility has also been found in the *Aedes scutellaris* group of mosquitoes⁴ and a rickettsia-like microorganism has been reported from a related species, *A. aegypti*¹².

We have found a rickettsia-like microorganism, possibly *Wolbachia pipientis*, in eggs from incompatible and normal crosses of *Culex pipiens*. It is found in the cytoplasm of the eggs and other cells of the mosquito but not in mature sperms. The presence of the microorganisms in the maternal cytoplasm and their transovarial transmission suggests that they might be the cause of cytoplasmic incompatibility.

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JANICE HARUMI YEN
A. RALPH BARR

School of Public Health,
University of California,
Los Angeles

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BOOK REVIEWS

Old World Revisited

Old World Monkeys (Evolution, Systematics and Behaviour). Edited by J. R. Napier and P. H. Napier. Pp. xvi+660. (Academic Press: New York and London, 1970.) £9.50.

THIS volume contains nineteen contributions from a symposium in Burg-Wartenstein (July 1969), sponsored by the Wenner Gren Foundation. The primary intention was provision of a basis for revising the systematics of Old World monkeys (superfamily Cercopithecoidea), and that aim has been admirably fulfilled. The papers provide an excellent source of up to date information on a number of key topics. In particular, it is invaluable to find in one volume a review of the fossil history of the Old World monkeys (Simons), a concise survey of biochemical aspects of monkey evolution (Barnicot and Wade; Sarich), an account of the oft neglected area of Sundaland (Medway), a series of papers on the behaviour of forest-living monkey species (Lowe's Guenon-Bourlière *et al.*; Drill-Gartlan; Gray Langurs-Ripley), a fascinating account of cross-species modification of social behaviour in baboons (Kummer *et al.*), and an annotated classification (Thorington and Groves). Among the behavioural papers, Struhsaker's account of the relevance of *Cercopithecus* vocalisations for classification is particularly noteworthy. Academically, the overall product is a highly useful and stimulating book, for which tribute should be paid to the sponsors and the editors. This book should prove to be essential for anyone concerned with Old World monkeys and their evolution.

Interest in biochemical aspects of Primate evolution is growing rapidly, and outsiders will find the balanced account provided by Barnicot and Wade both readable and informative. Evolution operates through modifications in the structure of DNA, and such changes can be directly expressed in protein structure. Ideally, an analysis of amino-acid sequences in various proteins should provide highly reliable data on evolutionary change. Actual sequencing is rare, however, and most workers estimate amino-acid change largely with electrophoretic techniques (Barnicot and Wade) or through immunological cross-reaction (Sarich). In spite of the limitations of such techniques, Sarich claims to be able to provide an

absolute evolutionary time scale, given calibration of *one* point from the fossil record. It is unfortunate that he should have selected an extremely questionable date (that of the divergence of Cercopithecoidea and Hominoidea) for calibration, and that he should have proceeded to a controversial dating for the divergence of apes and man, where the fossil record is comparatively good. As Thorington hints in his introduction, new techniques of this kind are best tested by comparison with existing classifications, rather than *vice versa*. Eventually, biochemical data will provide a precise tool for investigating evolutionary relationships; but there are still many difficulties of *interpretation* to be mastered. Nonetheless, it does emerge quite clearly from both biochemical contributions that the New World monkeys are probably not separately derived from prosimian ancestors; although the date of divergence is not clear, it seems obvious that there was a specific stock which gave rise to all the monkeys and the apes.

A review of the fossil history of the Old World monkeys has long been overdue, and Simons does much to clarify a somewhat confused situation. There is, however, one conspicuous omission. Simons states that *Apidium* should be classified within the Cercopithecidae, and he has also suggested that *Oreopithecus* is a close relative of *Apidium* (Simons, E. L., *Scientific American*, **211**, 50; 1964). Since this implies that *Oreopithecus* is an Old World monkey (contrary to Hürzeler's interpretation), adequate treatment of this fossil would have been welcome. Simons also gives the impression that available fossils are sufficient for zoogeographical interpretation, which is not the case. His apparent conflict with the views expressed in Napier's stimulating discussion of palaeoecology and evolution should be viewed in this light.

Typographically, this book does not do justice to its academic importance. The mixture of (sometimes inaccurate) typescript with neat printing is unpleasant, and it is disruptive to find the figures and tables grouped at the ends of the papers. There are many typing errors, one of which has led to the surprising statement in Ripley's paper that "The food of the grey langurs consists primarily of termite clay" (page 486). Even on the book jacket, there is a

reference to "ethologists" (?). Presumably, the justification for this layout could be that of economy; but such economy is not evident in the high price quoted. R. D. MARTIN

Drugs in Confusion

Drug Dependence. Edited by Robert T. Harris, William M. McIsaac and Charles R. Schuster Jr. (Advances in Mental Science II.) Pp. xiv+342. (University of Texas: Austin and London, November 1970. Published for the Faculty for Advanced Studies of the Texas Research Institute for Mental Sciences.) 95s.

IN spite of the title, this is a miscellaneous collection of papers given at the "second annual symposium sponsored by the Texas Research Institute of Mental Science". The date of the meeting is nowhere mentioned but from internal evidence it seems to have been in the first half of 1968. There are twenty-four contributions of varying quality, arranged under the general headings "Biological Aspects" (eight papers); "Pharmacological Aspects" (three); "Behavioral Aspects" (five); "Therapeutic Programmes for Drug Dependence" (five) and "Social Aspects" (three). If the first group is redistributed (drug dependence is a biological phenomenon), the last sub-divided into "Penal Aspects" and "Social Aspects", and one or two of the most obviously misclassified papers helped into more appropriate company, we get pharmacology, nine; behaviour, seven; treatment, two; law and punishment, four, and society, two.

This kind of "balance" is fairly representative of collections of this kind, and it may well be due in part to the difficulty of collecting hard data in soft areas; although it should be pointed out that "hard" is not necessarily synonymous with "reliable" or even "objective", and is seldom coincidental with "relevant". But of course the hard scientists always have the best of that argument—to paraphrase the little girl (or, some say, the old lady), they can and do claim "you can't tell if it's relevant if you don't give me a grant". Another kind of imbalance is more worrying, however, and it is hard to say if it is exaggerated or lessened by mixing scientists from different disciplines together in this way. I mean the trespassing of scientists,

sometimes eminent ones, into fields of which they are not landlords, nor even tenants, and where they describe the scene, enunciate laws, or give directives on the authority of their absentee reputation instead of the evidence before them. Is it my own prejudice that social scientists are less inclined to this kind of bad manners than basic ones? Probably; for example, the only piece of discussion (of which there must surely have been more than this) that the editors have thought worth including follows a paper by the assistant director for science of the United States Bureau of Narcotics and Dangerous Drugs; their purpose in doing so is perhaps to remind him that the high tension passing through the barbed wire does not respect the direction from which the cow approaches it.

On the other hand, the two "social" authors seem to me to grow in stature by putting their roots down firmly on their side of the fence. Helen Nowlis, cool and wise as ever, emphasizes that there are two separate drug problems—a medical problem and a people problem; that student drug use is a social problem that extends far beyond drugs; and that "to rely on presenting the effects of drugs on the body is not enough".

Dr Bueno, on the other hand, ends his paper "The Problem of Drug Addiction (*sic*) in Mexico", and indeed the book as a whole: "their lies are so clear to their hearts and senses that I fear they'd rather die than renounce them, if not treated with intelligence, so strongly do they persist in their doctrines and superstitions". One might be pardoned for thinking that he is referring to the current debaters about certain problems of drug dependence; in fact, he is quoting from an early seventeenth century *Manual for the Ministers to the Indians*. Perhaps we need a twentieth century "Manual for Professors ministering to Each Other".

C. R. B. JOYCE

Inside Plants

Introduction to the Fine Structure of Plant Cells. By Myron C. Ledbetter and Keith R. Porter. Pp. ix+188 (51 Plates). (Springer-Verlag: Berlin and New York, 1970.) 54DM; \$14.80.

LED BETTER and Porter have produced an outstanding collection of plant electron micrographs. Probably no other laboratory in the world could bring forward a group of such sustained high quality across such a wide range of material. Such atlases, which have now been emerging regularly from various laboratories for about seven years, conveniently chart the progress made in the techniques for the preparation of plant material. That plant techniques have now, in the best laboratories, caught up with animal preparative techniques, can be demonstrated by

comparing one of the first atlases (Stephen Hurry's excellent *The Microstructure of Cells* of 1964) with this production.

The text, however, which is generous compared with most atlases and well referenced, has limitations. The highly critical approach, applied to the quality of the micrographs, has not been applied to the text. Several statements are either wrong or misleading, for example, "Apparently the S₂ layer has a higher content of matrix substances (pectins, lignins and *sporopollenins*) . . ." (page 61); "The cuticle . . . is in turn impregnated with . . . waxes and oils, which . . . render it resistant as well to insect pests and the invasion of microbial and viral diseases" (page 109); "The large size of mesophyll cells is due in part to their relatively large vacuole" (page 133); "The absence of starch from the plastids reflects only the inability of permanganate to fix it . . ." (page 161); ". . . the Ubsch bodies, which are absorbed onto the surfaces of the pollen grains" (page 168).

Other statements may be true, but have no scientific backing to my knowledge, for example ". . . the plasmodesmata provide for the intercellular movements of large and small molecules . . ." (page 49); ". . . the Casparian strip assures that entering ions must pass through the plasma membranes of the endodermal protoplasts . . ." (page 81); "Cutin . . . Produced by epidermal cells, it moves through their primary walls and floods out over their exposed surfaces . . ." (page 113).

Plate 6.3 is labelled "Collenchyma". It is, however, a filament cell, at best a very atypical form of this type of cell and not representative in a number of ways. The structure of companion cells is given in detail, but no mention is made of the fact that they are absent in all gymnosperms, in a few angiosperms as well, and in all angiosperms in early stages of development. Cutin chemistry is much better understood than is suggested on page 113. Lignin (page 109) is an infrequent component of the epidermal cells of higher plants.

In short, the micrographs of this work and their references may be used as the most up to date and (with regard to the micrographs) the most technically accomplished of their kind available in a collected form. But the text must be treated with great care.

B. E. JUNIPER

Comfort of Clothes

Clothing: Comfort and Function. By Lyman Fourt and Norman R. S. Hollies. Pp. ix+254. (Dekker: New York, November 1970.) \$14.50; £6.90. This book is concerned with the interactions of clothing with the thermal balance between man's metabolic pro-

duction of heat and his exchange of heat with the environment. Readers interested in this subject will doubtless be familiar with the classic *Physiology of Heat Regulation and the Science of Clothing* by L. H. Newburgh (Saunders, Philadelphia and London, 1949). The present book discusses more recent work which has added to basic knowledge of the subject, and includes information derived from studies of the comfort properties of military clothing conducted in government research laboratories in the United States and elsewhere.

The book deals exclusively with the interaction of "normal" clothing as worn by the military and by civilians in everyday life with the transfer of sensible and evaporative heat between man and his environment. It does not deal with special clothing using auxiliary power for heating or cooling inside the clothing (such as space suits) nor with the mechanical properties of clothing (strength, durability and so on).

The first chapter discusses various quantities and units used in clothing comfort studies, and the second considers clothing as a quasiphysiological system, including discussion of the air content of fabrics and clothing systems and the effects of wind and body motion on the thermal balance. The third chapter consists of a discussion of clothing types for different parts of the body.

The next chapter, concerned with heat and moisture relations in clothing, describes test methods involving apparatus and human subjects. The latter aspect of such studies is treated in more detail in the fifth chapter. There is an account of various physical properties of clothing and clothing materials in relation to comfort, including among others air and water permeability, flammability, static electricity and radiant heat characteristics, in the sixth chapter. Next, natural and man-made fibres are compared with respect to the comfort properties of fabrics made from them, and in a short final chapter the authors discuss briefly current trends and developments involving new materials and finishes, new techniques and new concepts of clothing design.

This book is an extensive review of published works with 477 references. For this alone it is valuable and will be useful to people concerned with the comfort of clothing in spite of a few misprints and errors.

W. H. REES

The Seas are Changing

The Sea: Ideas and Observations on Progress in the Study of the Seas. Edited by Arthur E. Maxwell. Vol. 4: New Concepts of Sea Floor Evolution. Part 1: General Observations. Pp. xi+791. £15.25. Part 2: Regional Obser-

vations, Concepts. Pp. xii+664. £15.50. (Wiley-Interscience: New York and London, June 1971.)

THE third volume of *The Sea*, edited by Maurice Hill, was published in 1963 and dealt with the Earth beneath the sea. It provided, and still provides, an excellent introduction to the techniques, theory and fundamental results of marine geology and geophysics. However, within a few years of its publication the whole conception of the origin of the deep-sea floor had undergone a complete revolution, and those parts of the third volume that dealt with ocean crust structure and evolution began to look in need of refurbishing. Now we have the fourth volume, dedicated to the memory of Maurice Hill, and entitled *New Concepts of Sea Floor Evolution*, which takes the appropriate parts of the previous volume, and revises and expands them in the light of the new plate tectonics. The expansion is considerable. What was about 500 pages now occupies 1,455, split into three parts and bound as two books. The first book contains part I, "General Observations", which is essentially a revised and expanded version of the material in the third volume, introducing some new topics, such as petrology and seismology, and concentrating on techniques and global summaries of results. The second book is new material. Part II, "Regional Observations", consists of a number of regional syntheses covering different parts, but not all, of the deep ocean floor and continental margins. The articles generally combine results from several geological and geophysical techniques to arrive at statements about the structure and origin of the areas under discussion. Part III, "Concepts", contains two articles, by Vine and Hess, and by J. T. Wilson, which set the general and regional observations into the global framework of plate tectonics.

A volume like this must have two distinct aims, to interest active scientists with new syntheses and ideas, and to build up a fundamental framework, to act as an archival source for the future. The first aim is not well met here, chiefly because of publication delays. Each article is dated, and dates range from April 1968 to July 1969, with most in the range July 1968 to February 1969. Things have moved a bit since then, in particular with the arrival of results from the JOIDES drilling programme in the deep oceans, and with the realization of the importance of triple junction evolution in the geological history of plate margins. Thus only a few of the articles make an impact as original and new, though in this class must come the thoughtful article on the structure of the Pacific basin by Shor, Menard and Raitt (book 2), and that on the benthic boundary layer by Wimbush and Munk (book 1).

The second aim, though of less immediate interest, is particularly important in determining the life of a book, and this aim is much better satisfied. Many of the authors have taken a great deal of trouble in assembling references, in giving accounts of results published in widely scattered papers, and in drawing the results together to make a solid framework. This is particularly successful in the section on regional observations, where such papers as that on the Mediterranean by Ryan, Stanley, Hersey, Fahlquist and Allan, and that on Pacific boundary structure by Hayes and Ewing will be invaluable as sources of assembled basic information. The price of the two books, £30.75, will be a considerable deterrent to the private purchaser. My own advice would be to make sure that your library gets both books, while if you feel that you can afford only one, buy the second book, with parts II and III, rather than the first. On the other hand, if you get a chance to review a copy, do not hesitate, it is certainly time well spent.

J. R. CANN

Cambrian Collection

Cambrian of the New World. Edited by C. H. Holland. Pp. 456. (Wiley-Interscience: London and New York, April 1971.) £10.50.

THIS is the first volume of a series designed to describe the Lower Palaeozoic rocks of the world. As Professor Charles Holland puts it, the intention is to provide an up to date, yet reasonably circumscribed survey illuminated by the authors' own views but retaining some measure of common organization. If the series can maintain the standard of the first volume this aim should be achieved with success.

The book contains six contributions: an account of the Cambrian of the stable interior of the United States by Christina Lochman-Balk; descriptions of the Cambrian of the Great Basin to the west and of the Cambrian of the Appalachians and New England to the east by A. R. Palmer, together with three chapters which summarize the Cambrian of Canada and Alaska, by F. K. North, of the North American Arctic, by J. W. Cowie and of South America, by A. V. Borrello. They are all clearly presented accounts, written by authors of considerable experience. Judging from the bibliography the text seems to have been completed about two years ago, but there is much new and previously undocumented material, particularly on the Cambrian of the west of North America.

An attractive feature is the manner in which the authors hand on unpublished material to their successors and go out of their way to point out

the possibilities for future work which might lead to definitive syntheses. It is to be hoped that this approach attracts stratigraphical palaeontologists to the rich fields awaiting them. As Palmer remarks, as many as seven different biostratigraphical entities can be recognized in the Early Cambrian sequences of the Great Basin.

It goes without saying that a central theme in this book is the record of transgressions and regressions of the Cambrian seas over the Americas. At some time or another in the Cambrian, a part of every state in the Union was covered by a marine incursion. There are admirable syntheses of the different assemblages of rock which resulted and discussions of such topics as the possible influence of the underlying geological structures on the extent of these seas and repeated references to the interplay of biological activity and the kind of rock that resulted. Such discussions bring out the necessity to disentangle contemporary faunas which differ because they inhabited different environments from groups of animals which evolved through the passage of time and which might be used to define subdivisions of the Cambrian.

The reader is left with a clear picture of Cambrian events as revealed by present knowledge and with a sense of where progress is being made in current research and what the future might hold in store as investigations continue. Professor Holland and his colleagues can be congratulated on a fascinating book which I can recommend to any geologist interested in the Lower Palaeozoic history of our planet.

J. SUTTON

Physics and God

Proceedings of the International Conference on Thermodynamics held in Cardiff, UK, April 1-4, 1970. Edited by Peter T. Landsberg. (International Union of Pure and Applied Chemistry, in conjunction with the International Union of Pure and Applied Physics, and the Institute of Physics and the Physical Society. *Pure and Applied Chemistry*, Vol. 22, Nos. 3-4, 1970.) Pp. ix+215-555. (Butterworth: London, December 1970.) £9.

LET me say at the outset that I do not think that, purely academically, I am properly qualified to evaluate this book. I propose to review it, all the same, for the sake of interest and amusement: because it seems to me, as a modest but persistent user of commonplace thermodynamic methods in experimental physics, that this conference, which I did not attend, must certainly have been those things—and more. It illustrates the power of a simple macroscopic principle such as thermodynamics to

provide a magnificent variety of all things from the profoundly philosophic through the simply utilitarian to the distinctly risible. It is sometimes difficult to distinguish (if one wishes or ought to) between the first and last of these. Take, for example, the following exchange in the discussion reports concerning the direction of time.

D. Park—(Massachusetts, USA)—It seems that we get our sense of time direction very much more from the radiation of the Sun . . . than from anything the Universe is doing. Why on Earth should non-radiative living processes be bound up with the ultimate fate of radiation? This is not clear in Landsberg's statement, but Narlikar has his own answer. According to it, if suddenly the Universe started to contract, then it would seem to us that, as a result of distant events, the Sun would start re-absorbing radiation.

Landsberg—It would seem so to God, not to living things. God would say, ah, the Universe is contracting and everybody is getting younger while, I, God, am getting older.

Costa de Beauregard—No! Eternity is time-extended!

Landsberg—*Mon Dieu* (laughter)! I didn't really mean God.

What shall our undergraduates, struggling grimly uphill on their Carnot cycles, make of this? It was not, of course, a conference for undergraduates, but that is a legitimate question, and the answer is, I hope, that they should make much—in the sense that these are the sort of deep philosophical problems that stimulate people to study physics—at least they provided one of my own major motivations and I do not think, in spite of appearances, that things are so very much different now, fundamentally. It is a pity that the glamour of particle physics and expensive accelerators, and the hubris apparently inevitably engendered in some of their adherents, have distracted attention from the equally—or more—fundamental questions raised by less expensive disciplines. It is also anomalous (in a rational, not a political) sense in this age of relevance: a good deal of the work of this conference might well have application in industry and elsewhere now and in the foreseeable future.

Rather few reports of conference proceedings have more than a transient appeal, as a collection of papers of current interest in a special field. This is surely one of them, which should be widely used and be of lasting value. The section on foundations is perhaps difficult and esoteric and will be read mostly by thermodynamicists, but there is wide interest in, for example, phase transitions and surfaces. There are papers on the theory of fading memory, irreversibility and quantum mechanics,

astrophysics and relativity. There are papers and discussions on the teaching of thermodynamics. It is impossible to get an adequate idea of the scope and balance of the book, however, without examining it, which I very strongly recommend.

It seems a pity to carp, but I must say that this book is so valuable that an index would have been well worthwhile. It might have enabled me to detect some reference to information theory, which has so far escaped me. One other omission is the thermodynamics of finite systems. This state of matter, intermediate between non-interacting single-particle behaviour and the infinite thermodynamic limit, is surely of fundamental interest. It concerns, for example, nuclear matter, polymers, macromolecules and many biological systems, and could lead to a radical reassessment of our understanding. Not much is known experimentally in this area, but there is an awakening interest in the properties of small assemblies of particles, and this may well be a useful addition in a future conference.

D. F. BREWER

Information Science

Introduction to Science-Information Work. By C. W. Hanson. Pp. 199. (Aslib: London, June 1971.) £3; members £2.40.

ALTHOUGH information science has been developing rapidly during the past twenty years, and education in the field has been offered in the past ten years, there are still no definitive textbooks. It is a pity, therefore, that Mr Hanson, after his long pioneering experience at the British Scientific Instrument Research Association and at Aslib, as head of research, should now present a book on the mixed objectives of "information, documentation and library work" and called "science-information work". Information science is largely recognized as distinct from librarianship; in Great Britain, the term covers both practical and theoretical aspects, so that the expropriation of the term in the United States for theoretical studies of the science of information is not sufficient justification for abandoning it in Britain. Furthermore, information science is now extending into non-scientific fields, so that it is not just science-information. It was Mr Hanson who suggested the title "information scientist" in 1957.

Even as an "introduction" the book is quite elementary in content, although what it covers is most lucidly, carefully, and thoroughly described. It is in five parts. The first part deals with the problems of communication and the flow of information, the growth of the literature and the media of communica-

tion. The second part describes the primary and secondary types of literature, and non-documentary sources of information—organizations and people—a much-needed reminder in these days of mechanized services. There is then a rather short adumbration of mechanization, information retrieval and selective dissemination of information, all in a very general manner and in only seven pages. The fourth part discusses the provision of information to enquirers and users, and the making of searches for such information. Finally, there is some account of library operations, including lending, reprography and microforms in relation to information services; mechanized methods of library housekeeping are also considered. A short appendix gives attention to aspects of writing, editing, printing, publishing, translating and public relations.

In general, the book is centred on conditions in the United Kingdom, and foreign literature and sources of information are given scant attention. There is much sound commonsense in the advice given, but there are some unexpected flaws. The production of abstracts bulletins within industrial firms and research associations is not described. Patents are dismissed as unimportant as sources of information, although they frequently describe research which may not achieve wider publication for several years. To suggest that *International Critical Tables* (1927) is the last publication of its kind is too insular and ignores many excellent foreign publications.

The book is specifically addressed to school leavers, undergraduates and even older unqualified people, although information science is rapidly becoming a fully graduate profession. It deals too much with routine matters and makes the work seem rather a pedestrian activity. This is a pity, because the principal attractions of information science are the wide variety of tasks, the breadth of knowledge to be covered by keeping up to date and in touch with current research, and the contacts with people, from the factory floor to management. Increasingly there are opportunities to develop advanced computer-based systems, and there is a growing field of research in information science itself. Useful though this book may be at basic routine practical levels, it lacks the enthusiasm and the intimations of advanced work and problems which will inspire new entrants to the profession. Information departments, handling both technical information and management information, may well become the essential nerve centres of industry in the future. In the absence of the much needed advanced textbooks, even an "introduction" should point out, boldly, the way ahead.

J. FARRADANE

CORRESPONDENCE

Chemical Societies

SIR,—Your recent editorial about the Society of Chemical Industry (SCI) has just been brought to my notice (*Nature*, 231, 411; 1971) and, as I believe I was the only individual member to protest against the proposed merger of the SCI with the other chemical bodies, may I explain why I did so? Other protests came from members of one of the sections of the SCI (*Chemistry and Industry*, 1592, December 12, 1970).

I am a member of the Chemical Society (CS) and SCI, but not of the Royal Institute of Chemistry (RIC) because I do not satisfy the professional membership requirements.

The original proposals were for the amalgamation of the three bodies under a new unifying charter and, as long as this was the case, I was in favour and voted accordingly in the CS referendum. As long as a new charter was the ultimate aim, any divergence on the way towards it was not important, for it seemed certain that the lawyers advising the Privy Council would sort out anything which ought not to be allowed. Suddenly, however, in a document issued to members by the CS, what was to be the new CS became the "new" CS and it appeared obvious that amalgamation under a new charter was not contemplated within the foreseeable future. As none of the bodies have power in their charters to amalgamate, any other kind of amalgamation was, therefore, unlawful.

As far as the CS is concerned, I am now waiting to see the scheme which the Charity Commissioners must be drafting for the uniting of two charitable bodies (CS and the Faraday Society) with two which are not (RIC and the Society for Analytical Chemistry). Because there is no power to amalgamate in the charter of the CS, this scheme will show how it can all be done through new bye-laws alone, which themselves may be inconsistent with the charter (Council Members "appointed by" the RIC against "electing . . . other Members of Council" by the Fellows; Fellows of "any nationality" against "loving subjects"). It will also show how these new bye-laws are to be approved at a meeting which, somehow, took place on April 21, 1971, when a resolution was passed for the new bye-laws to come into force forthwith. It will then reconcile that with the supplementary charter which says that "no such resolution . . . shall become effective . . . until the expiration of one month" and will then reconcile the whole with the original charter, which declares "no resolution or Bye-law shall on any account . . . be made . . . in

opposition to the general scope, true intent and meaning" of the charter or of the laws or statutes of the realm (including the Charities Act, 1960?) and that if any such "rule or Bye-law shall be made, the same shall be absolutely null and void to all intents, effects, constructions, and purposes whatsoever".

How, in 1971, can the powers of the CS be so wide under its charter that it can amalgamate with (to be taken over by?) the RIC, when, in 1876, the "limited powers of the Chemical Society under its charter" were such that it could not accommodate within its own corporate identity that body of professional chemists which eventually became the RIC? (See *The Chemical Society 1841-1941*, pp. 52 and 53.) In any explanation, I hope account will be taken of the fact that the only thing which seems to have changed since 1876 is the addition of a supplementary charter in 1920 and that took away the class of associateship under which professional chemists might possibly have been accommodated.

In your editorial of November 14, 1970 (*Nature*, 228, 597; 1970), you mention the "enormous expense of negotiating a single" charter. Could not some of this expense have been avoided (anyway, for those bodies which are charities—the CS and SCI) by asking the Charity Commissioners to prepare a scheme under section 15 of the Charities Act? This provides machinery for amending, free of charge to the charity, the charters of those charities which are established by Royal Charter. The idea is partly to stop charities needlessly incurring legal costs when the Commissioners can do what is necessary without charge to the charity. Charity money should be spent on charitable objects and anything the commissioners do for free means that just so much more will be so spent.

Yours faithfully,

J. C. WILLIAMS

60A High Street,
Edgware
Middlesex HA8 7EJ

Smoking and Cancer

SIR,—It would be a pity if the somewhat personal nature of the exchanges between Dr John Higginson (*Nature*, 232, 355; 1971) and Dr T. D. Sterling (*Nature*, 231, 543; 1971), about the availability of unpublished data on smoking and lung cancer, were to obscure a real point of principle involved.

A scientific author basing his conclusions on the results of an experiment takes it for granted that he has to publish

enough information for the experiment to be repeated, and its results confirmed or not, as the case may be. In biology and medicine, and in the social sciences, conclusions may sometimes be drawn from observations which cannot be repeated, but here also the data ought to be made publicly available, to allow other interpretations. No useful scientific purpose is served by publishing unverifiable hypotheses; and that is so even if they are unverifiable only because the information needed for verification is kept private.

Ideally everything relevant ought to be published, but for reasons of space that may be impossible. As Dr Sterling has pointed out, this has not been done with most of the smoking/lung cancer investigations, and perhaps in other medical fields as well, where published conclusions have been derived from data which are not generally available, except by favour of the authors. This is unsatisfactory, since it is in no way to "impugn the integrity and competence of the numerous scientists and committees in many countries who have reviewed exhaustively the extensive data published on this subject" (if I may quote Dr Higginson) to accept that there is at least a remote chance that some of them may have been mistaken, and that there might be advantages in allowing a fresh look from an unprejudiced, or at any rate a different, point of view.

Granted that much of the data may be in a form unsuitable for publication in scientific journals, and that it would clearly be quite wrong to conclude that a busy and important medical scientist "who fails to make his data available to any Tom, Dick or Harry is lacking in credibility" (to quote Dr Higginson again), would it not be possible to have an arrangement whereby the data are deposited in some central library or institute and publicly available without reference to the author, to be copied and quoted from just as though they had been published with the original paper?

The British Museum (Natural History) has for many years operated such a scheme for biological and ecological data, which are often both voluminous and undigestible, and which journal editors are rightly reluctant to print, substituting instead a note to indicate where they are to be found in full. This seems to work well enough, and allows an author to refer to unprinted results without any fear of being accused of keeping them to himself, away from public investigation and criticism.

Yours faithfully,

C. B. GOODHART

University Museum
of Zoology, Cambridge

Announcements

University News

Dr C. D. Papaliolios has been appointed professor of physics in **Harvard University** and **Dr David K. Cohen** has been appointed professor of education and social policy.

Professor F. M. Arscott, University of Surrey, has been appointed professor of pure mathematics in the **University of Reading**, in succession to **Professor R. Rado**, on whom the title of professor emeritus has been conferred.

Dr H. P. Rang, University of Oxford, has been appointed to the chair of pharmacology in the **University of Southampton**.

Dr W. M. Hutchison has been appointed to a personal professorship of parasitology in the **University of Strathclyde**.

Miscellaneous

The sixth annual **Karl G. Jansky lecture-ship** of the National Radio Astronomy Observatory, Charlottesville, Virginia, has been awarded to **Professor Charles H. Townes**, University of California.

The **Horace Le Marquand and Dudley Bigg research fellowship** has been awarded by the council of the Royal Society to **Dr G. R. Shellam**, Commonwealth Serum Laboratories, to enable him to study the immune response to tumour specific antigens at the Tumour Immunology Unit, University College London.

Special merit promotions have been awarded to thirty scientists and technologists employed in government and other public establishments: *Civil Service*: **D. Küchemann**, Royal Aircraft Establishment; **P. S. Bulson**, Military Vehicles Engineering Establishment; **J. F. Gittins**, Services Electronics Research Laboratory; **T. P. McLean**, Royal Radar

Establishment; **R. J. Murgatroyd**, Meteorological Office; **D. W. Robinson**, National Physical Laboratory; **J. W. C. Gates**, National Physical Laboratory; **E. L. Goldsmith**, Royal Aircraft Establishment; **J. K. Lancaster**, Royal Aircraft Establishment; **K. R. May**, Microbiological Research Establishment; **R. L. Moss**, Warren Spring Laboratory; **J. B. Mullin**, Royal Radar Establishment; **M. E. Peover**, National Physical Laboratory; **P. B. J. Crisp**, Quantity Surveying Department. *Agricultural Research Council*: **F. Brown**, Animal Virus Research Institute; **C. G. Butler**, Rothamsted Experimental Station; **L. E. A. Rowson**, Unit of Reproductive Physiology; **A. Feinstein**, Institute of Animal Physiology; **J. W. B. King**, Animal Breeding Research Organization; **D. L. Lee**, Houghton Poultry Research Station; **W. G. Siller**, Poultry Research Centre. *Natural Environment Research Council*: **J. C. Swallow**, National Institute of Oceanography; **A. Watson**, Nature Conservancy. *Science Research Council*: **B. E. J. Pagel**, Royal Greenwich Observatory; **A. H. Gabriel**, Astrophysics Research Unit; **N. M. King**, Rutherford High Energy Laboratory; **P. F. Smith**, Rutherford High Energy Laboratory. *UK Atomic Energy Authority*: **R. Bullough**, Atomic Energy Research Establishment; **A. Gibson**, Culham Laboratory; **R. S. Nelson**, Atomic Energy Research Establishment.

Errata

In the article "Gamma Rays (250 keV–2.3 MeV) from NP 0532" by **L. E. Orwig**, **E. L. Chupp** and **D. J. Forrest** (*Nature*, 231, 171; 1971), the following corrections should be made. The end of paragraph 4 should read "... to account for the drift of the pulsar period as a result of the slowdown of the pulsar, the Earth's rotation, the Earth's orbital motion and

the motion of the balloon". Paragraph 5, line 6, should read " ± 0.5 ms", and line 4 should read "Another source of error ...". Paragraph 6, line 3, should read "15 h 50 m 30 s UT ...", and line 7 should read "15 h 50 m (29.9956 $\pm$ 0.0003) s ...".

The headline for the article "Formation of New Connexions in Adult Rat Brains after Partial Deafferentation" by **P. D. Wall** and **M. D. Egger** (*Nature*, 232, 542; 1971) should read "Mapping of the receptive fields of cells in the thalamus and cortex after destruction of a dorsal column nucleus in the rat reveals evidence of functional reorganization in the central nervous system." The penultimate line of the legend to Fig. 1 should read "... the axes show distances".

PROFESSOR D. GWYNNE EVANS was mistakenly referred to as **Professor D. Gwynne** in University News in *Nature*, 232, 508 (1971). Professor Evans is the director designate of the Lister Institute, as well as its professor of bacteriology and immunology.

British Diary

Wednesday, September 1

133rd Annual Meeting (eight days) British Association for the Advancement of Science, at Swansea.

Multivariable Control System Design and Applications (4th UKAC control convention, three days) Institution of Electrical Engineers, at the University of Manchester.

Thursday, September 2

518th Meeting (two days) Biochemical Society, at the University of Sussex, Falmer, Brighton, Sussex.

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